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Cover photo: Male harvestman, *Stygnoplus clavotibialis* (Opiliones: Laniatores: Stygnidae), on Mount Saint Benedict in Trinidad. The two bulbous structures are the second segments of the chelicerae. Photo by Bryan E. Reynolds.

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Redescription of *Diplocentrus zacatecanus* (Scorpiones: Diplocentridae) and limitations of the hemispermatophore as a diagnostic trait for genus *Diplocentrus*

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Abstract. The scorpion *Diplocentrus zacatecanus* Hoffmann (1931) was originally described as a subspecies of *Diplocentrus keyserlingi* Karsch 1880 on the basis of six syntypes and was later elevated to species level. We designate a male lectotype and redeseribe the species, including illustrations of the hemispermatophore of a male collected near the type locality. In this genus, the hemispermatophore is poorly sclerotized and lacks elaborate capsular structures, which are taxonomically useful in other genera. We review the variability in the hemispermatophores of males from one population, including five comparisons of the right and left hemispermatophores of the same males. Our results showed asymmetry in the length of the right and left hemispermatophores of the same individual. We also observed the presence of "crenulations" or "spines" in two different hemispermatophores (not complementary ones). We conclude that caution should be used when describing the hemispermatophore of only one male and considering it as diagnostic for the species, because of the high levels of intraspecific variation.

Keywords: Diagnostic character, hemispermatophore, lectotype, taxonomy

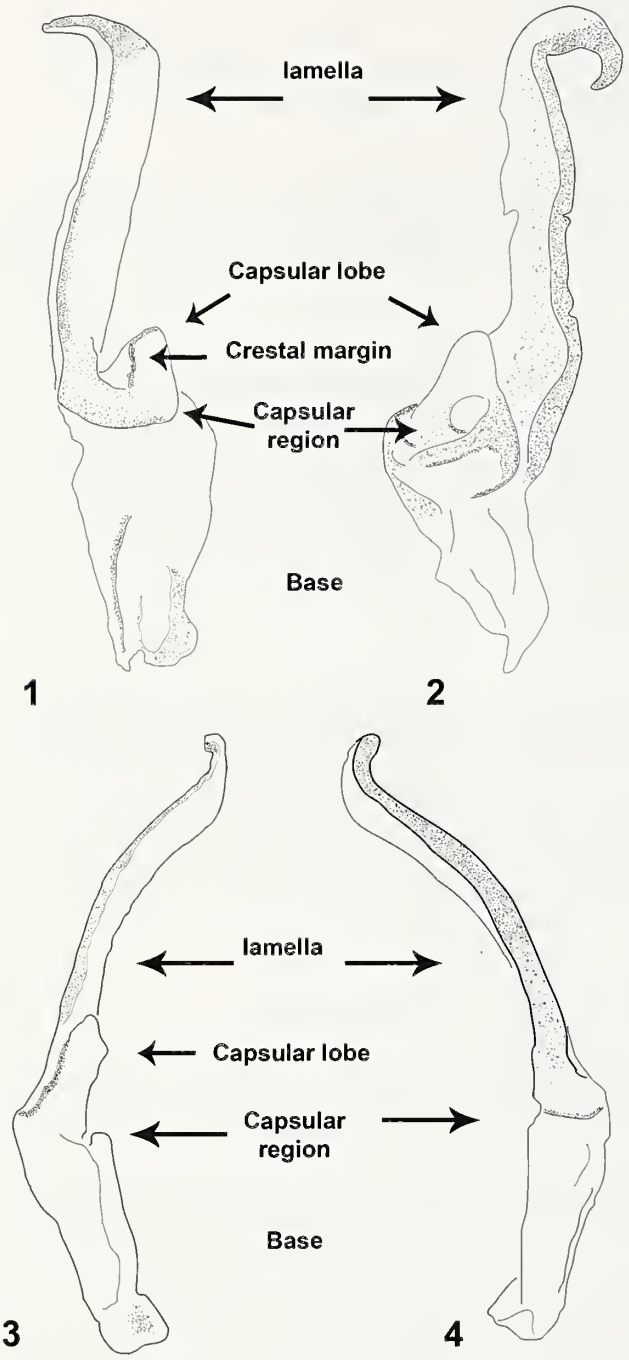
Hoffmann (1931) described three subspecies of *Diplocentrus keyserlingii* Karsch 1880 from Mexico. The nominate subspecies, *D. keyserlingii keyserlingii* Karsch 1880, was originally described from Oaxaca, but Hoffmann (1931) erroneously assigned specimens from Hidalgo to this taxon. The second subspecies, *D. keyserlingii tehuacanus* Hoffman 1931, was described from Tehuacan, Puebla, and the third, *D. keyserlingii zacatecanus* Hoffman 1931, was described based on six specimens from Tepezala, Aguascalientes.

Francke (1977) elevated *D. keyserlingii tehuacanus* to species level and redescribed it based on the holotype. Stahnke (1981) redescribed *D. keyserlingii* and designated a lectotype from Oaxaca. Sissom & Walker (1992) utilized *D. zacatecanus* as a specific epithet but provided no formal nomenclatural or taxonomic indication to justify the change of status. Subsequently, Sissom (1994) formally elevated *D. zacatecanus* to species level based on a comparison with *D. keyserlingii*. Most recently, Ponce *et al.* (2009) enlarged the known geographic distribution of *D. zacatecanus* to include the states of Aguascalientes, Durango, Guanajuato, Hidalgo, Mexico, Michoacan, Queretaro, San Luis Potosi and Zacatecas. However, researchers have undertaken no other taxonomic work on the species. In the present contribution, *D. zacatecanus* is redescribed based on a male lectotype designated herein.

In scorpions, various structures of the hemispermatophore have been used as species-specific diagnostic characters in several genera from different families (e.g., Vachon 1952; San Martín 1963; Koch 1977; Lamoral 1979; Stockwell 1989; Maury 1980; Sissom 1991, 1994a; Williams & Savary 1991; Acosta & Ochoa 2001; Soleglad & Sissom 2001; Ojanguren-Affilastro 2005; Ojanguren-Affilastro & Ramirez 2009; Peretti 2003, 2010; Ochoa & Prendini 2010; Prendini 2006; Prendini & Esposito 2010; Prendini et al. 2006; Francke & Ponce-Saavedra 2010; Santibáñez-López & Francke 2010; Botero-Trujillo & Flórez 2011; Mattoni et al. 2012). However, in other cases, as reported by Jacob et al. (2007) in at least one species complex of the genus *Euscorpius* Thorell 1876, the hemispermatophore may prove useless in separating species.

The hemispermatophore of species within the genus *Diplocentrus* Peters 1861 is of lamelliform type (sensu Francke 1979, Figs. 1–4); it has a simple capsule with a capsular lobe just above it, where "spines" in its margin have been illustrated. (However, the terminology referring to this lobe in the literature is inconsistent: see taxonomic comments below). No sclerotized mating plugs have been described similar to those found in other families such as Vaejovidae, which have proven useful as diagnostic characters (see species description of the genus *Vaejovis* of the "*eusthenura* group," e.g., Santibáñez-López & Sissom 2010); nor are there complex lobes with spiny projections as in the family Bothriuridae (e.g., Ojanguren-Affilastro 2005). Sissom & Wheeler (1995) proposed some differences between the hemispermatophores of three species of *Diplocentrus* as follows: "Hemispermatophores of the three species show some potentially important differences. The hemispermatophore of *D. spitzeri* has a very slender distal lamina that tapers distally and has a distinctly crenulated dorsal margin of the median capsular lobe; that of *D. williamsi* typically has a relatively broad distal lamina and the dorsal margin of the median lobe is weakly crenulated; while that of *D. peloncillensis* bears a very slender distal lamina with a feebly granular dorsal margin on the median lobe." Later, in the "Variation" section of the same work, both authors recognized that a study of hemispermatophore variability is necessary before their full value (or lack thereof) in diplocentrid systematics can be established (Sissom & Wheeler 1995).

The hemispermatophores of at least 17 species of the genus *Diplocentrus* have been described and illustrated (see Stockwell 1988; Sissom 1994; Francke & Ponce-Saavedra 2005; Santibáñez-López & Francke 2008). All reports focused on the presence or lack of "spines", or "crenulations" on the dorsal edge of the capsular lobe. Otherwise no attempt has been made to describe the hemispermatophore in detail and to analyze intraspecific variation. Hence, an additional goal of



Figures 1–4.—Diagrammatic description of the structures present in the hemispermatophores of *Diplocentrus* scorpions. 1. Dorsal view; 2. Ental view; 3, 4. Lateral views. The dorsal margin of the crest on the capsular lobe is the most sclerotized part of the hemispermatophore and it can be crenulated, smooth, or serrated (variation is found, see Figure 12 and text).

this study was to analyze the hemispermatophore morphology of *D. zacatecanus* in order to establish its usefulness as a diagnostic character for the species. We also aimed to explore additional potential taxonomic characters in the genus.

METHODS

Taxonomy.—Nomenclature and mensuration follow Stahnke (1970), except for trichobothrial terminology after Vachon (1974), and metasomal and pedipalpal carinal terminology after Francke (1977). Surfaces of the pedipalp, carapace, mesosoma, and metasoma were observed under UV light, as in Santibáñez-López & Sissom (2010). Higher-level taxonomy of scorpions follows Coddington et al. (2004) and Prendini & Wheeler (2005). Photography of the female and male carapace, pedipalp femur, patella, and chela under ultraviolet light is according to Prendini (2003) and Volschenk (2005).

Specimens.—The species was redescribed from a lectotype chosen from the syntype series deposited at Universidad Nacional Autonoma de Mexico. Intraspecific variation on pectinal tooth counts and on telotarsal spiniform setae was analyzed on five adult males and five adult female topotypes, along with 15 adult males and one adult female from a different population. Other specimens studied listed below were deposited at the Colección Nacional de Arácnidos, Instituto de Biología, UNAM (CNAN), and at the American Museum of Natural History (AMNH).

Hemispermatophore study.—A series of 45 adult males was collected 1 km SE of Nuevo Alamos, Queretaro, in May 2010. The males were found at night with ultraviolet lights, roaming on the surface in search of females (one couple was found engaged in the courtship dance), and thus all the adult males were judged to be ready for mating and with fully-formed hemispermatophores. The hemispermatophores of ten males from that sample were analyzed as indicated below. Dissections followed Vachon (1952) and Sissom et al. (1990). Three different procedures were followed to clean hemispermatophores: a) manual cleaning, b) immersion in clove oil and c) digestion of the soft tissues of the paraxial organ in pancreatin (as in Álvarez & Hormiga 2007). If manual cleaning is conducted appropriately and carefully, it can be effective. However, if not done properly, manual cleaning can destroy potential structures due to the poor sclerotization of this structure (as it has been described in Francke & Ponce-Saavedra 2005; Santibáñez-López & Francke 2008; Santibáñez-López et al. 2011). Immersion in clove oil reveals the borders of the structures of the hemispermatophore without destroying the surrounding tissues (i.e., the paraxial organ, where the hemispermatophore is formed); however, clove oil hardens those tissues and photographic results are poor. Finally, pancreatin digests soft tissues without causing any apparent damage to sclerotized structures (Álvarez-Padilla & Hormiga 2007); for this reason, it was the preferred method.

Hemispermatophore terminology follows mainly San Martín (1963). We took photographs of the hemispermatophores at the UNIBIO laboratory of photography, at the Instituto de Biologia, UNAM, with a Leica DFC490 camera attached to a Leica Z16 APO-A microscope, and layers were processed with the Leica Application suite program. We took measurements, given in millimeters, with an ocular micrometer calibrated at 10×.

Intraspecific variation: In order to observe intraspecific variation in this structure and to analyze potential diagnostic characters in the same, first we dissected five males and extracted the two hemispermatophores in order to analyze bilateral symmetry or the lack thereof. Secondly, another five males were dissected, but only one hemispermatophore was extracted from each (three right and two left) in order to obtain a larger sample for the comparative analysis. We took

Figures 5–6.—Habitus of the lectotype male of *Diplocentrus zacatecanus* Hoffmann 1931. 5. Dorsal; 6. Ventral.

measurements as follows: Total length (base length plus lamella length), lamella length (from the tip to the capsular region), base length, capsular region width and median lobe depth. The mean, standard deviation and coefficient of variation were calculated for all the structures measured.

Interspecific variation: We selected measurements of six species of *Diplocentrus* from the literature to compare against those from *D. zacatecanus* to observe potential diagnostic characters.

SYSTEMATICS

Family Diplocentridae Karsch 1880
Genus *Diplocentrus* Peters 1861
Diplocentrus zacatecanus Hoffmann 1931
(Figs. 5–10)

Diplocentrus keyserlingii zacatecanus Hoffmann 1931:317–319; Guijosa 1973:145, 150; Francke 1975:116; Vasquez & Zaragoza 1979:583.

Diplocentrus zacatenus (*lapsus calami*): Sissom 1986:256.

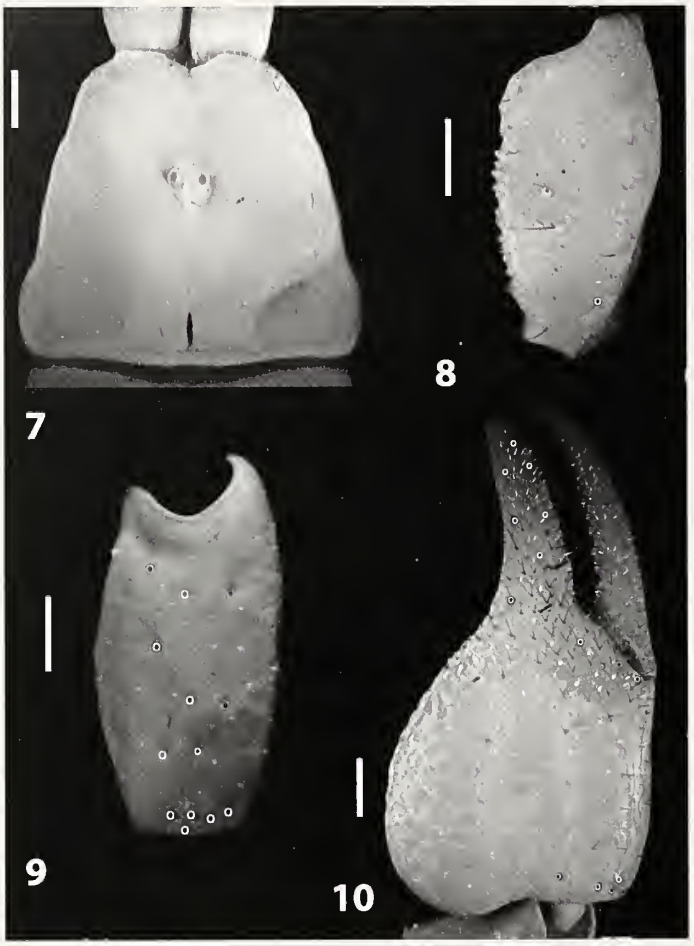
Diplocentrus zacatecanus: Sissom & Walker 1992:130; Sissom 1994b:265; Kovarik 1998:131; Sissom & Fet 2000:344; Kamenz & Prendini 2008:11, 42; Ponce et al. 2009:57–60; Contreras-Félix & Santibáñez-López 2011:62–63.

Diplocentrus keyserlingi (sic) f. *zacatecanus*: Beutelspacher 2000:29.

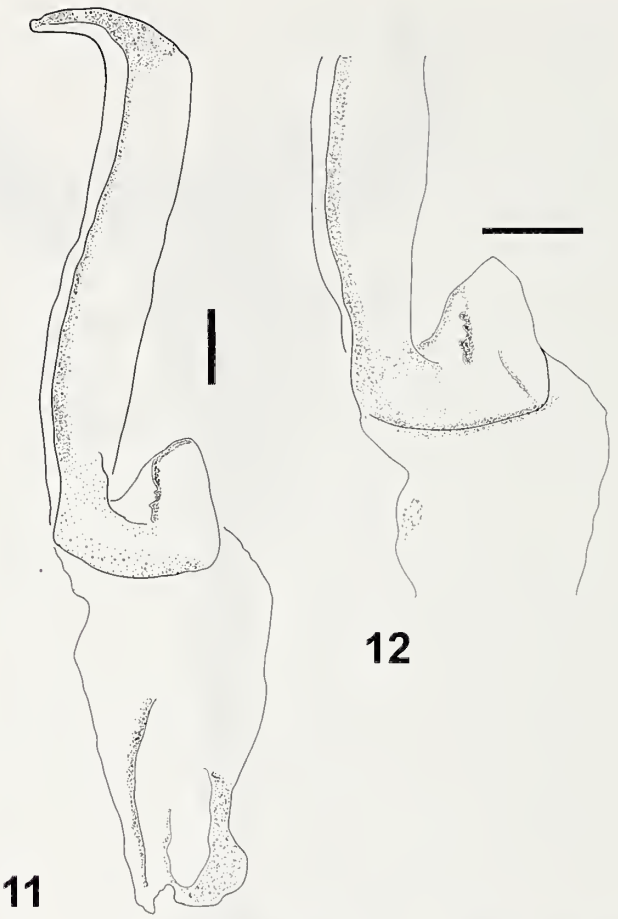
Type specimens.—Lectotype male, MEXICO: *Aguascalientes*: Tepezala (22°13.362′N, 102°10.014′W, 2100 m.), no date, no collector provided (CNAN-T0761). Paralectotypes: 2 males and 1 female (CNAN-T0762), collected with lectotype.

Other material examined.—MEXICO: *Aguascalientes*: Tepezala 1 km N (22°14.348′N, 102°10.467′W, 2048 m), 4 July 2005, O. Francke, J. Ponce-Saavedra, M. Córdova, A. Jaimes, G. Francke and V. Capovilla, 4 males (CNAN-S03075), 2 males (AMNH). *Zacatecas*: Road Sombrerete-Durango, km 179 (23°40.798′N, 103°41.712′W, 2448 m), 9 August 2005, O. Francke, W.D. Sissom, C. Lee, K. McWest, L. Jarvis, C: Dúran, H. Montaño and A. Ballesteros, 3 females (CNAN), 3 females (AMNH).

Diagnosis.—Adults 50 to 60 mm long. Orange brown to reddish brown. Carapacial anterior margin weakly granulose, median notch shallow, V-shaped. Pedipalp femur wider than deep, dorsal surface flat to slightly convex at the middle portion, sparsely granulose medially. On adult males, pedipalp patella dorsal external carina weak, smooth; ventral submedian carina faint; chela digital carina moderate, smooth; dorsal surface moderately reticulate. On females, pedipalp carination weaker and smoother. Basitarsi III and IV without prolateral and retrolateral subterminal spiniform setae. Telotarsal formula: 5/6: 5/6: 6/7: 6/7. Pectinal tooth count on males 12–17 (mode = 13); on females 11–13 (mode = 11–12) as reported by Ponce et al. (2009) from a sample of 71 males and 41 females.



Figures 7–10.—*Diplocentrus zacatecanus* lectotype male photographed under UV light. 7. Carapace dorsal view; 8. Femur, dorsal view; 9. Patella, external view; 10. Chela, dorsoexternal view. Scale bars = 1 mm. White circles highlight trichobothrial positions.



Figures 11–12.—Hemispermatophore of a topotype male of *D. zacatecanus*. 11. Dorsal view; 12. Detail of the capsular region, showing crenulated crest on capsular lobe. Scale bars = 0.5 mm.

Diplocentrus zacatecanus is similar to *D. tehuacanus* in size. The median notch on the anterior margin of the carapace is V-shaped in both species; the femur is wider than it is deep, and both species share similar pectinal tooth counts. However, *D. tehuacanus* has a lower modal telotarsal formula at 4/5:5/5–6:6/6:6/6; on adult males, the pedipalp patella dorsal external carina is obsolete on *D. tehuacanus*, whereas on *D. zacatecanus* it is weak and smooth; on males the chela is rounded in *D. zacatecanus*, whereas on males of *D. tehuacanus* it is slender.

Diplocentrus zacatecanus also resembles *Diplocentrus gertschi* Sissom & Walker 1992 from Nayarit. Both species share a pedipalp femur wider than deep and a similar telotarsal formula. However, *D. zacatecanus* can be clearly distinguished from *D. gertschi* by the punctations on the pedipalps (not present on *D. zacatecanus*). *D. gertschi* adults are colored dark brown, whereas adults of *D. zacatecanus* are reddish brown to orange brown. *D. zacatecanus* is close geographically to *Diplocentrus whitei* (Gervais 1844), sharing similar pedipalp femur proportions (wider than deep). It differs from *D. whitei* by its lighter body coloration (adult *D. whitei* are dark blackish-brown); a higher telotarsal formula in the last two legs in *D. whitei* at 7/8:7/8; by contrast, in *D. zacatecanus* it is 6/7:6/7; and higher pectinal tooth counts are found on males (18–20) and females (14–16), whereas in *D. zacatecanus,* they are 11–17 on males and 11–13 on females.

Description of male lectotype.—*Coloration:* Carapace light brown to pale yellow (old specimen in alcohol), venter pale orange to brown. Pedipalps orange to reddish brown, carinae darker. Mesosoma brown to medium yellow, venter pale brown. Metasoma light brown to orange. Telson orange to reddish brown, uniformly infuscated. Legs pale brown to pale yellow, uniformly infuscated.

Prosoma: Anterior margin "V" shaped, notch shallow, sparsely setose, weakly granulose (Fig. 7). Three pairs of lateral eyes, subequal in size. Carapaeial surface shagreened to minutely granulated towards the lateral surfaces.

Mesosoma: Tergites I–VI granulose towards the sides, shagreened at the middle portion. Tergite VII surface shagreened to weakly granulose toward the sides. Sternites III–VI weakly and faintly punctated. Sternite VII with submedian and lateral carinae weak to moderate, crenulated to slightly granulated. Pectinal tooth count: 13–14.

Metasoma: Ventral submedian carinae: on I–II moderate to strong, granulated; on III weak to moderate, granulated; on IV weak to faint, smooth. Ventral lateral carinae: on I–II moderate to strong, crenulated to slightly granulated; on III weak to moderate, crenulated; on IV weak to faint, smooth. Lateral inframedian carinae: on I strong, with large conical granules; on II moderate, granulated; on III weak to faint, smooth; on IV

Table 1.—Measurements of lectotype male and "syntype" female of *Diplocentrus zacatecanus* from Tepezala, Aguascalientes, México. Abbreviations: L= Length, W= width, D= depth.

	Male Lectotype	Female
Total L	42.3	41.4
Carapace L	5.4	5.4
Carapace W	3.4	3.6
Mesosoma L	13.9	13.9
Pedipalp L	17.7	16.6
Femur L	4.3	4
W	1.9	1.8
D	1.5	1.5
Patella L	4.7	4.5
W	2	2
D	2.3	2.2
Chela L	8.7	8.1
W	2.7	3
D	4.8	4.5
Movable finger L	5	5.5
Fixed finger L	4	3.5
Chelicera L	4	3.5
W	1.3	1.3
Movable finger L	2.5	2
Fixed finger L	1.7	1.4
Metasoma L	18.5	17.5
Segment IV L	4	3.7
W	2.5	2.4
Segment V L	5.2	5
W	2.1	2.1
D	1.7	2
Telson L	4.5	4.6
Vesicle L	3.6	3.8
W	2.1	2.4
D	1.7	1.9

faint to obsolete. Lateral supramedian carinae on I–III weak to moderate, crenulated; on IV weak, smooth. Dorsal lateral carinae: on I–II weak, smooth with one or two granules distally; on III–IV weak to moderate, smooth. Segment V 1.2 times longer than pedipalp femur: ventral median carina strong, granulated, with large subconical granules; ventral transverse carina strong, formed by four large subconical granules; ventral lateral carinae strong, granulated with large subconical granules; lateral inframedian carinae faint to obsolete, smooth; dorsal lateral carinae faint to obsolete, smooth. Intercarinal spaces: ventrally on segments I–V smooth; dorsally on segments I–II shagreened, on III–V smooth; laterally on segments I–V smooth; however, on segment V weak punctuation may be appreciate under UV light only.

Telson: Smooth, with granules basally; subaculear tubercle strong, subconical. Vesicle width /length ratio 0.58.

Pedipalp: Orthobothriotaxy type "C"; pattern typical for the genus. Femur wider than deep (Fig. 8). Dorsal internal carina strong, granular. Dorsal external carina weak to moderate, basally granular and smooth, fading out distally. Ventral internal carina moderate, granulose, fading distally. Ventral external carina faint to obsolete. Dorsal face flat to slightly convex medially, central area sparsely granulose with small granules. Ventral face flat, smooth. Internal face densely granulose, with large strong granules.

Patella: (Fig. 9). Dorsal internal carina weak to obsolete, basal tubercle moderately strong, bifurcated. Dorsal median carina strong, smooth. Dorsal external carina weak, smooth. External carina weak, smooth. Ventral external carina faint to obsolete. Ventral median carina faint. Ventral internal carina weak, granular. Dorsal, external and ventral faces smooth. Internal face minutely granular.

Chela: (Fig. 10). Dorsal margin of manus moderately carinated, strongly granular. Digital carina moderate, smooth. Dorsal secondary carina and external secondary carina weak to moderate, smooth. Ventral external carina originating at external condyle of movable finger articulation, converging towards ventral median carina and fading distally, weak, smooth. Ventral median carina strong, smooth to slightly crenulated. Ventral internal carina weak to obsolete, smooth. Three internal carinae originating at the middle portion of chela, all forming a shallow longitudinal depression where chela flexes against patella, weak, smooth. Dorsal face moderately reticulated, external face weakly to moderately reticulated. Fixed finger base: dorsal face smooth, with dense setation, external face flat, internal face feebly concave. Fingers curved.

Legs: Prolateral faces of femora and tibiae smooth. Basitarsi III and IV without prolateral subterminal, retrolateral subterminal and ventral median spiniform setae (Santibañez-Lopéz et al. unpubl. data). Telotarsal spiniform setae formula: 5/6 5/6: 5/6 5/6: 6/7 6/7: 6/7 6/7.

Hemispermatophore: (Extracted from a male collected in 2005; see above) (Figs. 11, 12), 5.5 mm total length; lamellate, weakly sclerotized, lamella 3.3 mm long. Capsular region 1.5 mm wide. Capsular lobe narrow, smooth; no other structures present.

Table 2.—Measurements (mm) of the hemispermatophores of *D. zacatecanus*. Male identifications are given in code. Abbreviations L = length, W = width, D = depth, X = average, STD = standard deviation, CV = coefficient of variation.

Specimen	number	459	450	451	468	469	453	467	456	455	464	X	STD	CV (%)
Right	Hemispermatophore L	4.5	4	4	3.8	4.3	4.8	4.5	-	-	-	4.27	0.35	8.30
	Lamella L	3	3.1	2.8	2.5	2.8	3.1	2.7	-	-	-	2.86	0.22	7.79
	Capsular W	0.9	1	1.2	1.1	0.9	1	0.9	-	-	-	1.00	0.12	11.55
	Median lobe D	0.9	0.9	0.8	1	0.8	0.9	0.8	-	-	-	0.88	0.07	8.13
	Base	1.5	1.3	1.2	1.3	1.5	1.7	1.8	-	-	-	1.47	0.22	15.05
Left	Hemispermatophore L	5.4	4.2	4.1	3.9	4.2	-	-	4.4	5.1	4.3	4.45	0.52	11.71
	Lamella L	3.1	2.5	3.1	2.5	2.8	-	-	3	3.6	2.7	2.91	0.37	12.64
	Capsular W	0.9	0.9	1.1	1.3	1	-	-	1	0.7	1	0.98	0.17	17.64
	Median lobe D	0.8	0.8	0.8	0.8	0.8	-	-	0.7	0.9	0.9	0.81	0.08	10.28
	Base	2.3	1.7	1	1.4	1.4	-	-	1.4	1.5	1.6	1.54	0.37	24.07

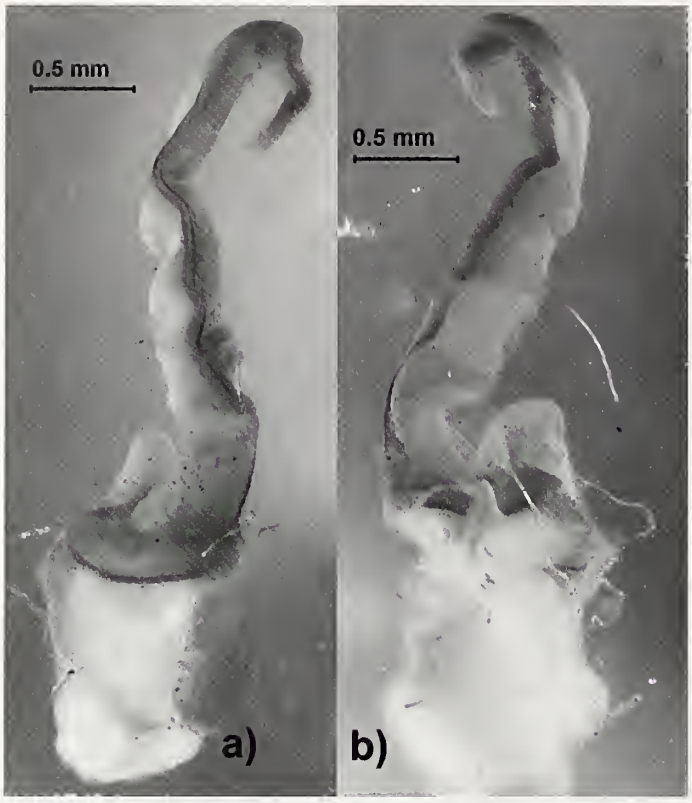


Figure 13.—Right hemispermatophore of male 451. a. Dorsal view; b. Ental view.

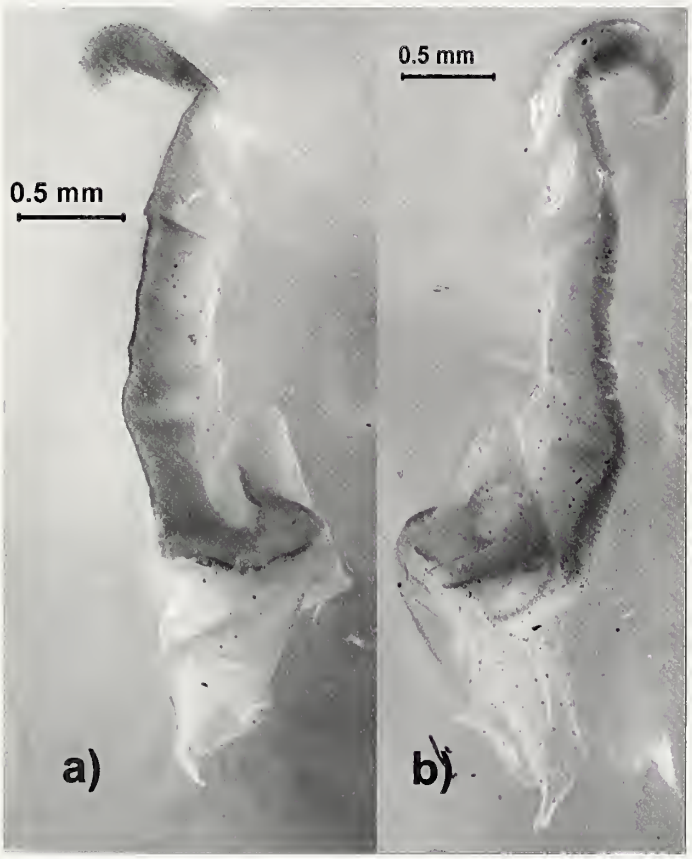


Figure 14.—Left hemispermatophore of male 451. a. Dorsal view; b. Ental view.

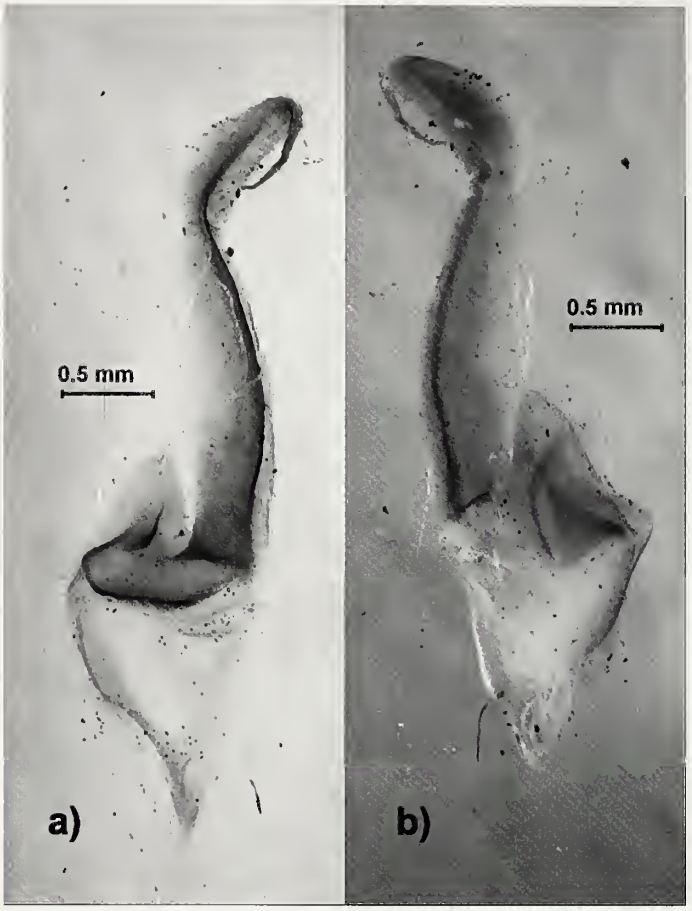


Figure 15.—Right hemispermatophore of male 469. a. Dorsal view; b. Ental view.

Variation (see also Ponce-Saavedra et al., 2009).—*Diplocentrus zacatecanus* exhibits reduced sexual dimorphism compared to other species in the genus. Female differs from the male in some measurements (Table 1) and as follows:

Mesosoma: tergites darker than on male. Pectinal tooth count lower: 11–13.
Metasoma: Carination moderately developed. Telson vesicle width/ ratio: 0.63.
Pedipalp: Chela rounder than on male, digital carina weak, smooth. Dorsal and external faces reticulated, but ridges are weaker than on male.

Pectinal tooth count on males (n = 30): 1 comb with 11 teeth (broken), 10 combs with 12, 3 combs with 13, 7 combs with 14, 6 combs with 15, 2 combs with 16 and 1 comb with 17 teeth. On females (n = 12): 4 combs with 11 teeth, 5 combs with 12 and 3 combs with 13 teeth. The typical telotarsal spiniform setae formula is: 5/6: 5/6: 6/7: 6/7. Telotarsal spiniform setal counts (n = 42):

Leg I prolateral: 1 tarsus with 4 setae, 38 tarsi with 5 and 3 tarsi with 6 setae.
retrolateral: 2 tarsi with 5 setae, 33 tarsi with 6 and 7 tarsi with 7 setae.
Leg II prolateral: 27 tarsi with 5 setae and 15 tarsi with 6 setae.
retrolateral: 24 tarsi with 6 setae and 18 tarsi with 7 setae.

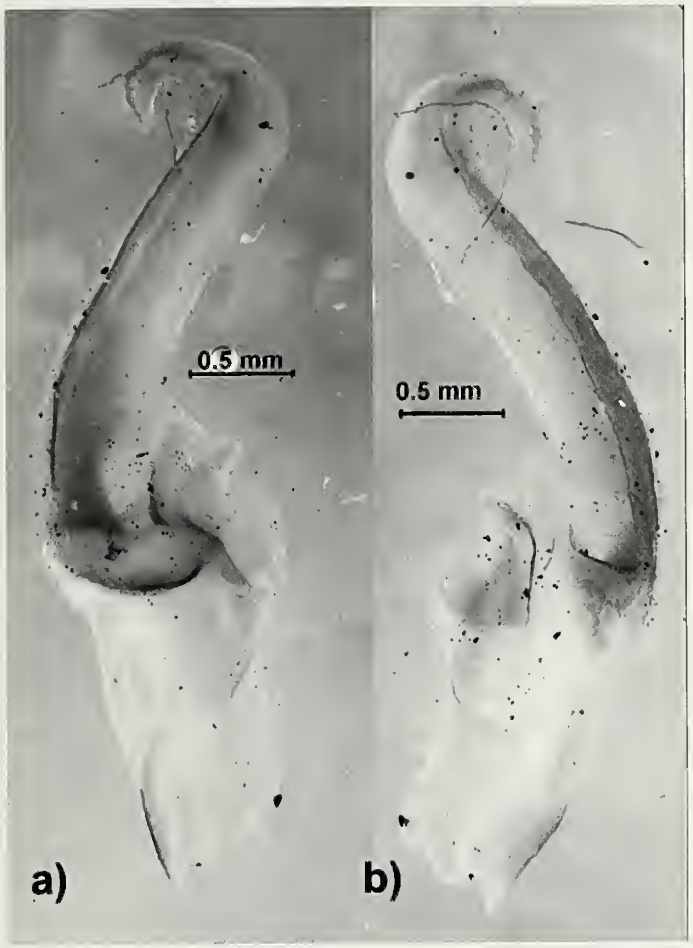


Figure 16.—Left hemispermatophore of male 469. a. Dorsal view; b. Ental view.

Leg III prolateral: 3 tarsi with 5 setae, 32 tarsi with 6 and 7 tarsi with 7 setae.
retrolateral: 7 tarsi with 6 setae, 33 tarsi with 7 and 2 tarsi with 8 setae.
Leg IV prolateral: 35 tarsi with 6 and 6 tarsi with 7 setae.
retrolateral: 2 tarsi with 6; 30 tarsi with 7 and 2 tarsi with 8 setae.

Distribution.—Aguascalientes, Estado de Mexico, Durango, Guanajuato, Hidalgo, Michoacan, Queretaro, San Luis Potosi and Zacatecas (see Ponce-Saavedra et al. 2009).

Analysis of the hemispermatophore in *Diplocentrus zacatecanus*.—*Intraspecific variation:* Measurements of the 15 dissected hemispermatophores showed differences in the total length of the hemispermatophore (Table 2). We found that these differences resulted from the length of the base and not the length of the lamella, because the coefficient of variation in the former is almost twice as large as in the latter (Table 2). The base of the hemispermatophore is often damaged during extraction.

Asymmetry between the right and left hemispermatophores of the five completely dissected specimens is shown in the first five columns of Table 2: all measures of the right hemispermatophore were less variable than the left one. On average, the right hemispermatophore is shorter than the left; however, this could be the result of poor preservation or damage of the

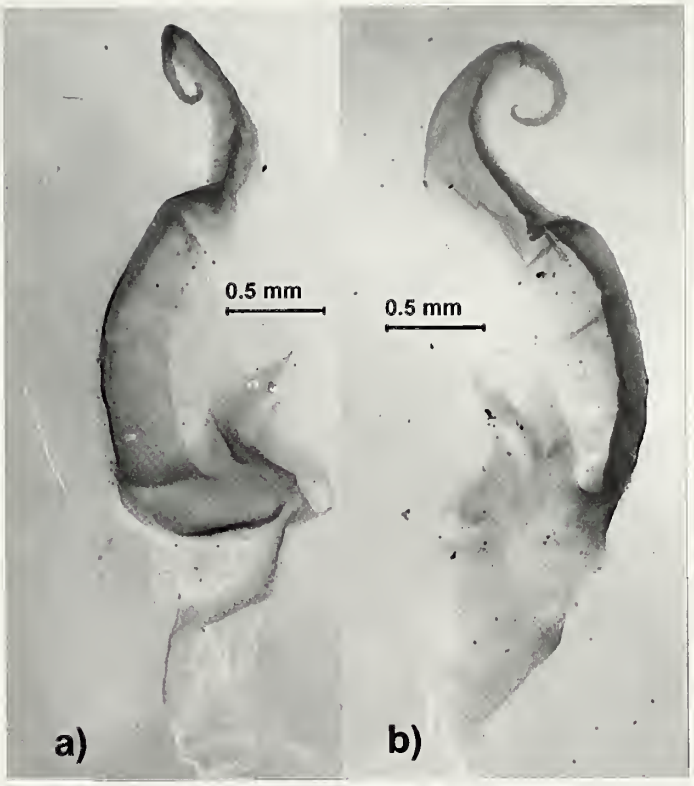


Figure 17.—Right hemispermatophore of male 464. a. Dorsal view; b. Ental view.

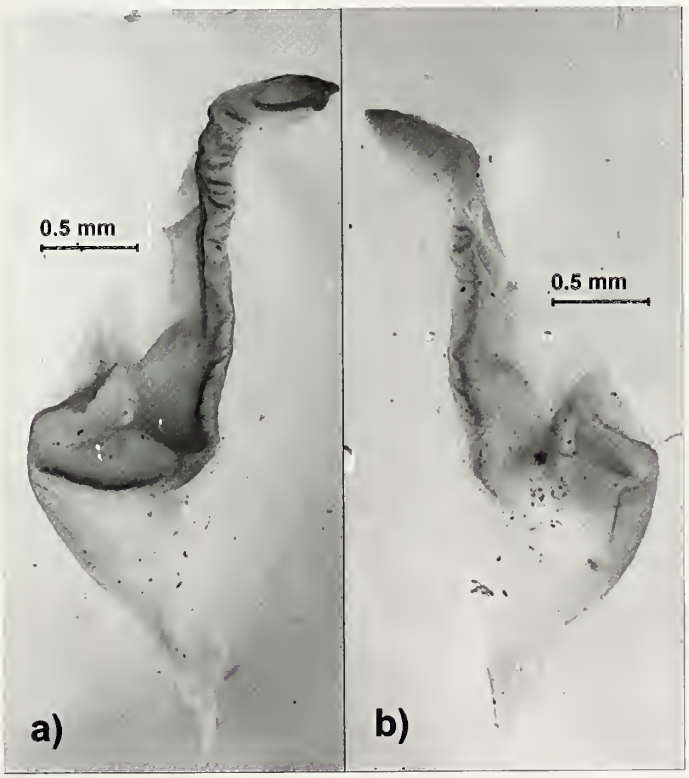


Figure 18.—Left hemispermatophore of male 464. a. Dorsal view; b. Ental view.

Table 3.—Selected ratios of the hemispermatophore of males of *D. zacatecanus*. Abbreviations L = length, W = width, D = depth. X = media, STD = standard deviation, CV = coefficient of variation.

	450		464		451		468		469								±	CV
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	453	467	456	455	459	X	STD	%
Total L / lamella L	11.1	13.3	14	13.5	12.4	13.7	16.5	16.5	13.4	13.4	13.4	16.9	13.6	11.8	12.6	13.7	1.7	12.4
Carapace L / lamella L	1.5	1.8	1.85	1.79	1.65	1.82	2.2	2.2	1.79	1.79	1.81	2.25	1.81	1.7	1.74	1.85	0.21	11.4
Mesosoma L/lamella L	4.14	4.97	5.19	5	4.45	4.93	6.08	6.08	4.82	4.82	5	6.29	5.37	4	4.48	5.04	0.68	13.5
Pedipalp L/lamella L	4.58	5.5	5.7	5.5	5.32	5.89	6.84	6.84	5.93	5.93	5.45	7.13	5.56	5.27	5.45	5.79	0.68	11.7
Chela L/lamella L	2.33	2.8	2.89	2.79	2.77	3.07	3.48	3.48	3	3	2.84	3.75	2.89	2.67	2.84	2.97	0.36	12
Chela W/lamella L	0.72	0.87	0.89	0.86	0.9	1	1.16	1.16	0.93	0.93	0.84	1.13	0.93	0.87	0.84	0.93	0.13	13.6
Chela D/lamella L	1.31	1.57	1.74	1.68	1.61	1.79	1.96	1.96	1.68	1.68	1.55	2	1.59	1.5	1.65	1.68	0.19	11.1
Mesosoma L/capsular W	21.3	21.3	14.7	14	12.6	11.5	11.7	13.8	13.5	15	9.12	16.8	8.06	12	15.4	14.1	3.73	26.5
Chela D/capsular W	6.71	6.71	4.95	4.7	4.55	4.17	3.77	4.45	4.7	5.22	2.82	5.33	2.39	4.5	5.67	4.71	1.19	25.3
Chela W/capsular W	3.71	3.71	2.53	2.4	2.55	2.33	2.23	2.64	2.6	2.89	1.53	3	1.39	2.6	2.89	2.6	0.64	24.5

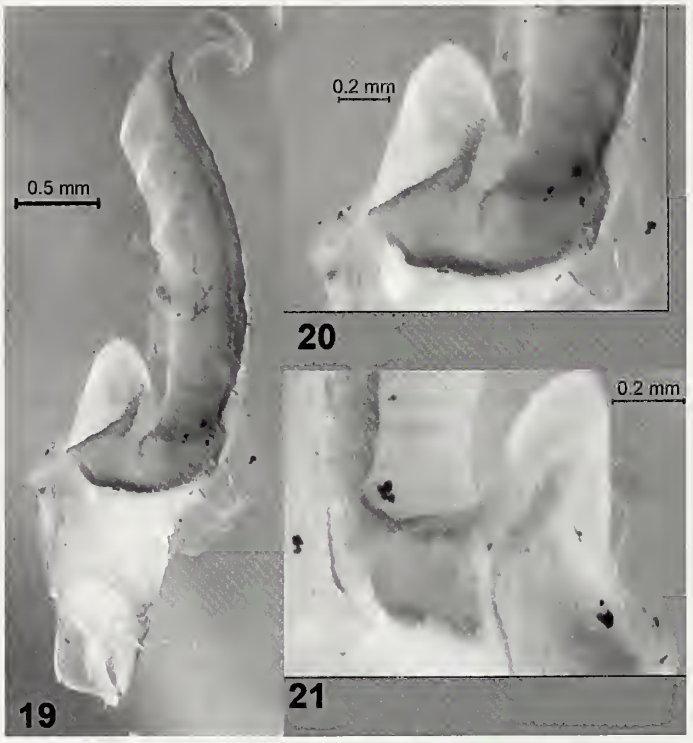
base during extraction. Only one specimen showed considerable bilateral asymmetry in length (right hemispermatophore of specimen 459 is shorter than the left one: Table 2). The lamella of the right hemispermatophore is shorter than the left one on specimen 450; however, its base is shorter, resulting in a similar total length.

The right hemispermatophore lamella tip of specimen 451 was curlier than the left one (see Figs. 13, 14); the right lamella tip of specimen 469 was more slender than the left one (Figs. 15, 16). The right lamella tip of specimen 464 was the curliest of all the hemispermatophores analyzed, whereas the left one was wide and planar (Figs. 17, 18). The chela depth / lamella length ratio (coefficient variation percentage) showed minimum variation, whereas the chela width and capsular region width ratio was more variable (Table 3). No distinct, sclerotized structures were found inside the capsular area; the ectal capsular lobe is distinct and weakly sclerotized, does not form a distinct crest or ridge, and is without crenulations or other ornamentation (Figs. 13–21).

The crenulated margin of crest at the median lobe was observed on only one of the two hemispermatophores of two different specimens of *D. zacatecanus* from the Queretaro population (and in the topotype male from Aguascalientes as well); however, it was missing on the other 13 specimens studied. Therefore, we report for the first time considerable variation in this structure, both bilateral and between-individual asymmetry of the same population. This crenulated margin has been described or observed from at least 10 species in the genus *Diplocentrus* (see Stockwell 1988; Sissom 1986; Francke & Ponce-Saavedra 2005; Francke 2007); nevertheless, most of them were observed on the single hemispermatophore illustrated, and thus no information on intraspecific variation is available for those species. Since this crenulation was variable even in paired hemispermatophores (i.e., the same spermatophore), caution should be taken to examine more than one specimen when it is described or used to compare taxa.

Interspecific variation: Hemispermatophore ratios of six different species were compared to *D. zacatecanus* (Table 4). There may be a relationship between the scorpions' total body length / hemispermatophore length. *Diplocentrus bicolor* possesses a proportionately smaller hemispermatophore compared to its large body, whereas *D. steeleae* has a proportionately larger one compared to its small body. The hemispermatophore of *D. coddingtoni* Stockwell 1988 has a relative wider capsular area compared to its total length, whereas *D. poncei* Francke & Quijano & Ravell 2009 possesses a more slender capsular area compared to its total length. However, all these comparisons should be taken lightly, due to the high variation in corresponding measurements (length and capsular width) reported above in *D. zacatecanus*.

The hemispermatophore provides phylogenetic (therefore diagnostic) information at the family level. However, our study revealed that, at least for one species within the genus *Diplocentrus,* the hemispermatophore is highly variable in size and capsular lobe sculpturing and thus does not offer useful diagnostic characters at this level. We suggest that the hemispermatophore should be ignored as a taxonomic character within *Diplocentrus* when new species are described, because it



Figures 19–21.—Left hemispermatophore of male 459. 19. Dorsal view; 20. Detail of the capsular region, dorsal view; 21. Detail of the capsular region, ental view.

Table 4.—Morphometric ratios of the hemispermatophores of seven species of the genus *Diplocentrus*. All measurements were taken from the literature, except for *D. zacatecanus*, which is from this study.

	Total body length / hemispermatophore length	Hemispermatophore length / capsule width	Capsule width / median lobe depth	Reference
D. coddingtoni	15	2.5	1.5	Stockwell (1988)
D. steeleae	4.17	6	1.43	Stockwell (1988)
D. poncei	7	11.11	1.13	Francke and Quijano-Ravell (2009)
D. churumuco	8.75	5	0.8	Francke and Ponce-Saavedra (2005)
D. bicolor	10.87	5.75	1	Contreras-Félix and Santibáñez-López (2011)
D. tenango	6.23	4.92	1.27	Santibáñez-López and Francke (2008)
D. zacatecanus	16.52	3.45	1.38	This study

lacks significant structures in the capsular region and because it is highly variable. However, additional studies are needed to evaluate the taxonomic value of the hemispermatophore at the generic and subfamilial levels within the Diplocentridae.

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Freya ambigua (Araneae: Salticidae) introduced to the continental United States, with new synonyms

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Abstract. *Euophrys ambigua* C.L. Koch 1846 is again transferred, becoming *Freya ambigua*, COMBINATION RESTORED. This jumping spider species, native to northern South America, has been found in North America in the southern parts of two of the states of the USA: Florida (Broward, Hillsborough, Manatee, Miami-Dade, and Pinellas counties) and Texas (Cameron and Hidalgo counties). Previously known from Colombia, Suriname, Trinidad and Tobago, and Venezuela, it is now also recorded from Brazil and French Guiana. Two other names are reported as NEW SYNONYMS: *Menemerus fannae* Peckham & Peckham 1896 and *Freya perelegans* Simon 1902. A lectotype is designated for *Menemerus fannae*. The female of *F. ambigua* is described for the first time.

Keywords: Florida, jumping spider, South America, systematics, Texas

Freya ambigua (C.L. Koch 1846) is a medium-sized jumping spider that was originally described from Suriname. Another species from the same greater geographic area, *Freya perelegans* Simon 1902, was originally described from Venezuela. The latter species was also reported from Trinidad and Tobago (Cutler & Edwards 2002). Comparing our respective research endeavors on these two species, we have discovered that the two names represent one more widely distributed species. Subsequently, a third species, *Plexippus* (*sub Menemerus*) *fannae* (Peekham & Peckham 1896) from Colombia, has been found to be another synonym, further widening the geographic range of this speeies. The female apparently has not been described previously under any of these names, although it would not be surprising to find it has been described under yet another old name.

Starting a little more than a decade ago, there have been three records (Dixon & Coile 1999, 2000; Dixon & Anderson 2010) of females of this species reported from Florida. Since the female was neither described nor could these specimens be conclusively matched with a known male, a species name was not applied to this introduction, and it was known as *Freya* sp. Species identification awaited the collection of a male, which was only recently forthcoming. In the meantime, records from Texas indicated possible multiple exports of *F. ambigua* from its native range. A review of *F. ambigua* was initiated to establish a better understanding of its natural range and to clarify its nomenclature.

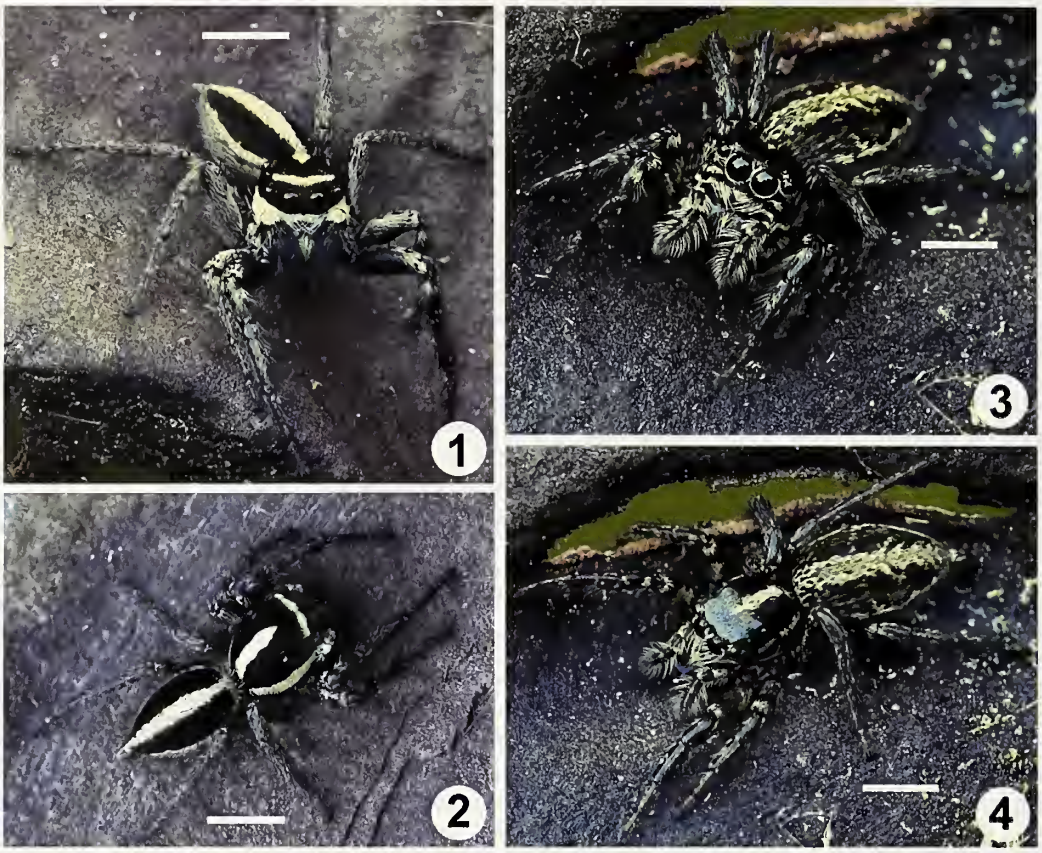
METHODS

Records identified by GBE from Florida were originally obtained by Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI), Bureau of Plant and Apiary Inspection personnel, and published in the DPI technical report, Tri-ology. These were supplemented with subsequent surveys by GBE with Lyn and Brooks Atherton. All DPI records are in the Florida State Collection of Arthropods (FSCA), along with further records from arthropod surveys of various Neotropical areas. Texas records were from the Texas A & M University Insect Collection (TAMUIC), and photographic records from this state were obtained through the courtesy of Dick Walton. Records from Brazil were identified by GRSR from the Museu Paraense Emílio Goeldi (MPEG), Belém and Instituto Butantan (IBSP), São Paulo. Records from the Brazilian states of Amazonia and Roraima were based on examination of photographs of specimens collected by Thierry Gasnier and Bruno R.S. Machado; these specimens should be deposited in the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, but have not been located by the staff. Additional material was examined from the United States National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C. GRSR provided the drawings and examined some types.

The lectotype of *F. perelegans* is located in the Muséum National d'Histoire Naturelle (MNHN), Paris, France. It was previously selected from the syntype series, redescribed, and illustrated by Galiano (1963). Other type specimens were provided for examination by the following institutions: Museum of Comparative Zoology (MCZ), Cambridge, Massachusetts; Museum für Naturkunde (ZMB), Berlin, Germany; and Naturhistorisches Museum Basel (NMB), Switzerland.

Specimens preserved in 70% ethanol were examined with a Leica MZ16A stereomicroscope. Male left palps were dissected at the coxa-trochanter joint and examined under high magnification for evaluation of details on the embolus and RTA. Epigynes were examined and illustrated in situ in ventral view. For details of inner structures, the epigynal plate was dissected and immersed in clove oil, cleared, and illustrated. Illustrations were done with the aid of a camera lucida on 'eggshell' paper with pen and pencil. Dissected parts were placed in microvials in the same vial with the specimen from which they were removed.

Abbreviations are used for the following morphological structures: AER = anterior eye row, AEW = anterior eye row width, ALE = anterior lateral eye, AME = anterior median eye, BL = body length, AOB = anterior ocular band, CH = carapace height, CL = carapace length, CW = carapace width, EFL = eye field length, I-II-III-IV = leg pair number starting from the anterior, PEW = posterior eye row width,



Figures 1–4.—*Freya ambigua*, living specimens from Tierra Verde, Florida. 1, 2. male; 3, 4. female. Scale = 2.0 mm.

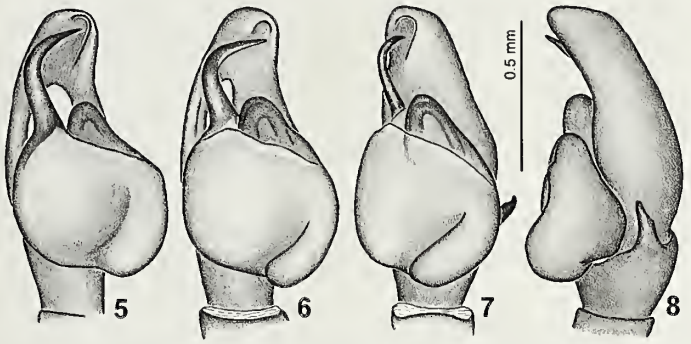
PLE = posterior lateral eye, PME = posterior median eye, RTA = retrolateral tibial apophysis (male left palp always illustrated). Measurements (in mm) were made of five specimens of each sex and are given as a range: minimum (mean) maximum. Nomenclature follows Platnick (2012). Exclamation marks with citations indicate type(s) were examined. Some specimens collected as juveniles were reared to adult (as indicated).

TAXONOMY

Order Araneae
Family Salticidae
Genus *Freya* C.L. Koch 1850

Relationships and diagnosis of *Freya*.—This genus belongs to an as yet informally defined neotropical group, the 'freyines' (Maddison et al. 2008), which is presently under study (G.B. Edwards in prep.). Although many putative genera in this group appear to have conspicuous colors and markings (e.g., *Nycerella* Galiano 1982, *Phiale* C.L. Koch 1846), *Freya* is one of a smaller number of genera that primarily have a cryptic color pattern, especially in females (e.g., Figs. 3, 4). A cryptic pattern in this context is considered to be a dorsal opisthosoma with a pale median stripe (sometimes modified with spots or chevrons) bordered laterally by a broad dark stripe on each side, usually brown (sometimes black or dull red, often with small pale markings), and with a venter consisting of numerous dark speckles on a pale background. Other genera that also have a cryptic pattern inelude *Kalcerrytus* Galiano 2000, *Sumampattus* Galiano 1983 and *Trydarssus* Galiano 1995.

Freya and similar genera are medium-sized jumping spiders (5–8 mm body length). The species of *Freya* can be characterized by a moderately slender, usually unmodified embolus originating on the prolateral side of the bulb (or if distal, without a bifurcate tip), lacking a conductor, usually with a lamella (fused or lost in a few species, but present in close relatives of those species where it is lost), and a robust simple RTA. The epigynal copulatory openings of *Freya* species are anterolateral or anterior without atria (or with a hooded atrium), and the copulatory ducts are short and broad. Of the other cryptic genera, *Freya* seems more closely related to *Kalcerrytus*, which also has the embolus originating prolaterally, but the latter genus is very dark in color (lacking pale carapace markings present in the other genera) with an incomplete median opisthosomal stripe, the RTA is frequently complex, and the epigynal copulatory openings are situated in atria that are not modified to cover the openings, but also have broad if somewhat more elongate ducts. *Sumampattus* and *Trydarssus* have the palpal embolus originating distally. *Sumampattus* has an embolus that is flared out and/or notched along its length, and lacks a lamella with the embolus, but has a prominent ventral membranous conductor and a robust simple RTA. *Trydarssus* has a lamella and lacks a conductor, but in addition the distinctive conical embolus is bifurcate at the tip, and the RTA is strongly bifurcate. Both genera have their epigynal copulatory openings anteromedial within larger exposed atria, and the copulatory ducts are moderately elongate and narrow.



Figures 5–8.—*Freya ambigua*, male left palp (from left to right): 5. holotype of *F. ambigua*, ventral view; 6. holotype of *Phiale albovittata*, ventral view; 7. same, retroventral view matching Galiano's (1963) illustration of *Freya perelegans*; 8. same, retrolateral view.

Type species.—*Freya* (*sub Euophrys*) *decorata* (C.L. Koch 1846)

Freya ambigua (C.L. Koch 1846), COMBINATION RESTORED
Figs. 1–13

Euophrys ambigua C.L. Koch 1846:206, f. 1253 (Dm)! *Freya a.*: C.L. Koch 1850:66.

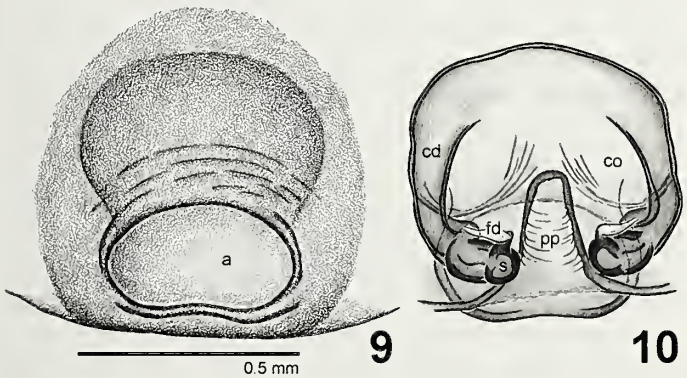
Menemerus fannae Peckham & Peckham 1896:74, pl. 6, f. 6 (Dm)! NEW SYNONYMY. Lectotype male designated (male with both palps attached), second male is paralectotype (palps detached).

Thotmes f.: F.O.P.-Cambridge, 1901:241, pl. 20, f. 22 (m). *Freya perelegans* Simon 1902:414 (Dm). NEW SYNONYMY.

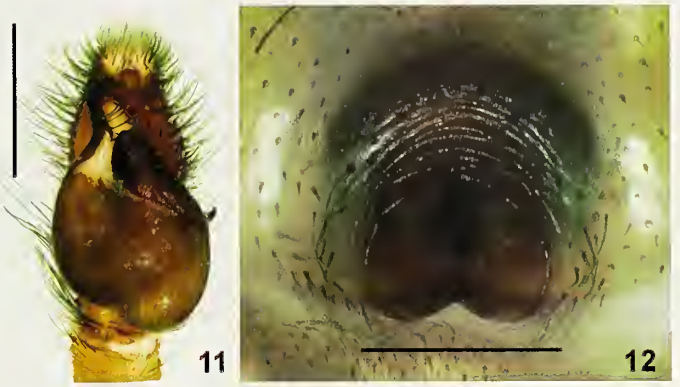
Plexippus f.: Petrunkevitch 1911:695. *Phiale albovittata* Schenkel 1953:51, f. 45a-b (Dm)! *E. a.*: Roewer 1955:1179. *F. p.*: Galiano 1963:359, pl. XX, f. 8-9 (m).

F. p.: Cutler & Edwards 2002:42. *F. p.*: Ruiz & Brescovit 2007:648 (S) [synonymized *P. albovittata* with *F. perelegans*].

Type material.—Holotype male *Euophrys ambigua*, SURINAME: no other locality data (Cordua, ZMB 1799); 3 syntype male *Freya perelegans*. VENEZUELA: *Distrito Capital*: Caracas (E. Simon, MNHN) [lectotype designated by Galiano (1963)]; holotype male *Phiale albovittata*. VENEZUELA: *Falcón*: Distrito Acosta, El Pozón, October 1924–January 1925 (K. Widenmeyer, NMB 2254); 2 syntype male



Figures 9–10.—*Freya ambigua*, epigyne of female from Pará (Brazil): 9. ventral view; 10. dorsal view, cleared. Abbreviations: a) atrium, cd) copulatory duct, co) copulatory opening, fd) fertilization duct, pp) posterior pocket, s) spermatheca.



Figures 11–12.—*Freya ambigua*, photos of specimens from Ruskin, Florida: 11. male palp, ventral view; 12. female epigyne, ventral view. Scale = 0.5 mm.

Menemerus fannae. COLOMBIA (NEW GRENADA): ex. Keyserling coll. (Peckham coll. 445, MCZ), lectotype designated herein.

Other material examined.—*Previous records* [Florida records from Tri-ology; Trinidad and Tobago locality records were briefly reported by Cutler and Edwards (2002) without other data, included here]: TRINIDAD AND TOBAGO: *Trinidad*: Caroni Co., Chaguanas Ward, Madame Espagnole River, Caroni Swamp, N of Cacandee Settlement, on mangrove, 20 August 1986, 1M (G.B. Edwards, FSCA); St. Andrew Co., Manzanilla Ward, Aripo Savannah, 23 August 1986, 1M, 1F (G.B. Edwards, FSCA); same location, 24 August 1988, 1F (C. Chaboo, FSCA); St. George Co., Northern Range, Arima to Blanchiseusse Rd., roadside at 300 m asl. on south side of range, 29 June 1999, 1F (G.B. Edwards, FSCA); San Rafael Ward, Arena Forest Preserve, vegetation in sandpit and old Caribbean pine plantation understory, 1 July 1999, 2M, 1F (B. Cutler, FSCA); same location and date, 1M, 2F (G.B. Edwards, FSCA); same location, 7 July 1999, 3M, 5F (G.B. Edwards, FSCA); Tunapuna, Mt. St. Benedict, secondary rainforest and pine forest, 4 July 1999, 1M (D.B. Richman, FSCA); same location, 8–9 July 1999, 1F (G.B. Edwards, FSCA); St. Patrick Co., Erin Ward, savannah vegetation, 2 July 1999, 1F (B. Cutler, FSCA); La Brea, Pitch Lake, weeds and grass near edge of pitch, 2 July 1999, 1M (G.B. Edwards, FSCA). USA: *Florida*: Manatee Co., Parrish, on Chinese water spinach (*Ipomoea aquatica* Forssk.), 7 October 1999, 1F (K. L. Etchells, FSCA); same location, at nursery, 15 November 2000, 1F (M.L. Runnals, FSCA); Miami-Dade Co., Medley, on mango (*Mangifera indica* L.), 15 December 2010, 1F (J.F. Revuelta, FSCA).

New Records: BRAZIL: *Amapá*: Oiapoque, Igarapé do Campo, 3.842°N, 51.855°W, 12 April 2005, 1M (Monteiro-Santos, MPEG 4996). *Amazonas*: Solimões River, between Coari and Manaus, 1F (T. Gasnier & B.R.S. Machado photographs of specimen collected by F.N. Rego & B.R.S. Machado, INPA, photos examined by GRSR). *Ceará*: Pentecoste, Fazenda experimental da UFC, 3.8°S, 39.35°W, pitfall traps in preserved Caatinga, August 2008–August 2009, 2M (R. Azevedo, IBSP 162857-162858). *Pará*: Juruti, Piranha, 2.210028°S, 56.122417°W, 14 May 2010–22 February 2011, 3M, 3F (N.A. dos Santos, B.V.B. Rodrigues & N.F. Lo Man

Figure 13.—Distribution map for *Freya ambigua*. Dots are records; circles in Colombia and Suriname represent records from those countries with no further data and are approximate.

Hung, MPEG 19195-19200); Belém, Campus de Pesquisa do Museu Paraense Emílio Goeldi, April 2012, 1M (G.R.S. Ruiz, MPEG 19201); Carajás, Serra Norte, Mina do Sossego, 6.445°S, 50.066°W, 23 February–6 March 2004, 1M (D.R. Santos-Souza, MPEG 4091); same location, 6.435°S, 50.069°W, 1F (E. Wanzeler, MPEG 4079); Altamira, Novo Progresso, 7.129°S, 55.425°W, 19–26 November 2005, 2M, 2F (D.R. Santos-Souza, MPEG 2676, 2679, 4395, 4396); same location, 19–24 November 2005, 2M, 2F (J.O. Dias, MPEG 2675, 2677, 4397, 4398); same location and date, 1M (D.F. Candiani, MPEG 2678); Castelo dos Sonhos, 8.217°S, 55.015°W, 16 November 2005, 1F (A.L. Nunes, MPEG 4394); same location and date, 2F (D.R. Santos-Souza, MPEG 4399); same location, 8.214°S, 55.020°W, 13 November 2005, 1M (A.L. Nunes, MPEG 2680); same location, 16 November 2005, 1M (A.A. Pinheiro, MPEG 2681). *Roraima*: Maracá, 1M (INPA, T. Gasnier & B.R.S. Machado photographs examined by GRSR). FRENCH GUIANA: *Kourou*: Montagne de Singes, disturbed primary forest, 17 May 2009, 1M (V. Vedel, FSCA); Route D13, near Guatemala, 13 April 1999, roadside savannah, 2F (G.B. Edwards, FSCA); *Roura*: junction Route N2 and Route de Bélizon, 3 April 1999, roadside, 1F (G.B. Edwards, FSCA); *Sinnamary*: near Malmanoury, 13 April 1999, roadside savannah, 1M (G.B. Edwards, FSCA). USA: *Florida*: Broward Co.: Dania Beach, John Lloyd State Park, Interdiction and Maritime Services survey, on sticky board, 8 October 2012, 1F (M.A. DaCosta & J. Garcia, FSCA); Hillsborough Co., Ruskin, beating salt grass [*Distichlis spicata* (L.) Greene], 6 April 2012, 1M, 2 subadults, 5 smaller juveniles (1M, 2F reared) (G.B. Edwards, L. & B. Atherton, FSCA); Miami-Dade Co., Miami, sticky trap on seagrape [*Coccoloba uvifera* (L.) L.], 18 June 2012, 1F (C. Pelegrin, FSCA); Pinellas Co., Tierra Verde, north shore of S. Duck Pond, 16 November 2011, 3M, 2F, 9 subadults (3M reared) (G.B. Edwards, L. & B. Atherton, FSCA); Ft. DeSoto Park, mulberry area in dense grass (1M on sapling persimmon), 16 November 2011, 2M, 1F, 2 subadults (1F reared) (G.B. Edwards, L. & B. Atherton, FSCA); same location and date, Soldier's Hole, on recumbent salt marsh aster, 1 subadult M (G.B. Edwards, L. & B. Atherton, FSCA). *Texas*: Cameron Co., Laguna Atascosa National Wildlife Refuge, 26.22375°N, 97.35454°W, 16–29 October 2008, FIT-ground, dense coastal brush, 1m (J. King & E. Riley 300, TAMUIC); Sabal Palm Grove Refuge, 25.84799°N, 97.41881°W, 18–31 October 2009, FIT-ground, palm forest, 1F (J. King & E. Riley 1433, TAMUIC); same location and trap type (Site 2), 25.84851°N, 97.41794°W, 31 October 2008–6 February 2009, 1M (J. King & E. Riley 435, TAMUIC); San Benito, 28 December 2011, 1 subadult M (T. Fuller photograph); Hidalgo Co.: pitfall trap in grass survey, 13–19 March 1981, 1F (FSCA); Bentsen Rio Grande Valley State Park, 26.17830°N, 98.38577°W, 4–17 October 2008, FIT-ground, cedar elm forest, 1M (J. King & E. Riley 280, TAMUIC); Estero Lano Grande State Park, 21 November 2011, 1M (R.K. Walton photograph); Lower Rio Grande Valley National Wildlife Refuge (LRGVNWR), La Coma (Site 1), 26.05302°N, 98.04665°W, 20 September–3 October 2008, pitfall trap, re-vegetated site, 1M (J. King & E. Riley 141, TAMUIC); same location and trap, 13–26 March 2009, 2M (J. King & E. Riley 618-621, TAMUIC); same location and trap, 24 April–7 May 2009, 1F (J. King & E. Riley 896-899, TAMUIC); same location and trap, 8–22 May 2009, 2M (J. King & E. Riley 976-979, TAMUIC); same location and trap type (Site 2), 26.05611°N, 98.03635°W, 4–17 October 2008, 1F (J. King & E. Riley 236-239, TAMUIC); LRGVNWR, McManus Unit, 26.05380°N, 98.04987°W,

2–17 November 2009, FIT-ground, ebony-guayacan association, 1F (J. King & E. Riley 1516, TAMUIC); Santa Ana Refuge, 18 May 1984, 1M (D.A. Dean, FSCA). VENEZUELA: *Bolivar*: 7.6 km SE Guasipati, 22 March 1982, 1M, 1F (G.F. & J.F. Hevel, USNM). *Guárico*: 44 km S. Calabozo Hato Masaguaral, 10 February 1986, 1F (R.B. Miller & L.A. Stange, FSCA). *Portugnesa*: Agua Blanca, rice field, 8 December 1982, 1M (J.M. Osorio, FSCA). *Vargas*: Tanaguarena, 26 December 1970, coastal building and garden, 1M (W. B. Peck, FSCA).

Range.—Northern South America from Colombia to French Guiana and northern Brazil. Introduced to the southernmost parts of the USA in Florida and Texas (Fig. 13).

Remarks.—Koch (1850) described *Freya* and correctly (based on knowledge available at that time) transferred his own species, *E. ambigna*, from *Euophrys* to *Freya*; however, Roewer (1955) reversed the transfer, thereby obscuring its relationship, since this caused the species to be associated with the wrong subfamily (Euophryinae). This species actually belongs to the "freyine" group of genera (Maddison et al. 2008).

There were three loose palps in the type vial of *Menemerus fannae*, even though only two males from "New Grenada" are indicated in the description. A matching pair of palps, which are very similar to the intact palps of the lectotype, has been placed with the paralectotype. The extra left palp matches the remaining right palp of a third specimen, noted in the original description from Guatemala. This palp was most likely misplaced during the original description, and it was returned to that vial. The Guatemalan specimen belongs to the related *Freya longispina* (F.O. P.-Cambridge 1901), originally described from Guatemala. As no records of *M. fannae* intermediate between the two localities exist, the distribution reported for this species, Guatemala to Colombia (Platnick 2012), is not supported. By inference (since *M. fannae* is a synonym), *F. ambigua* therefore is not known from Central America.

Since one of the three specimens in the original description of *M. fannae* is a different species, it is necessary to designate a lectotype, done above. This species was illustrated as having a red dorsum in Cambridge (1901), and reddish scales are still apparent on the dorsum of the two specimens from this locality; however, this may be due to severe fading or other preservation artifacts, as all other specimens from other localities have a mostly black dorsum, and the only reddish scales, if present, are around the eyes and the edges of some white markings on the carapace.

A few of the records reported from Trinidad and Tobago (Cutler and Edwards 2002) (the two Tobago localities and the Trinidad specimen from Mt. Zion) are either *Freya longispina*, previously cited only from Guatemala and Panama (Platnick 2012), or more likely a related species with an elongate RTA. These specimens are similar to, but do not quite match, the *P. fannae* specimen from Guatemala, or the description and illustrations of *F. longispina* in F.O.P-Cambridge (1901). This species will be referred to as *F. cf. longispina* in the Diagnosis. The records will be reported in detail elsewhere (Edwards in prep.).

Diagnosis.—*Euophrys ambigua* is transferred to *Freya* based on its cryptic color pattern as defined previously, the male having a simple hooked embolus originating prolaterally, lacking a lamella [although its close Central American relative *Freya bifurcata* (F.O.P.-Cambridge 1901), has a lamella], lacking a membranous conductor, and having a robust, simple RTA. The epigynal copulatory openings are anterolateral, and the copulatory ducts are broad and relatively short. In addition, the black dorsum with white stripes and bands of male *F. ambigua* (and its closest relatives) is quite similar to the black and white pattern of male *F. decorata*, the type species of the genus. The main difference between them is the pattern on the anterior dorsum of the carapace, which in *F. decorata* has three short stripes rather than a transverse band.

From its closest relatives, males of *F. ambigua* differ from males of the sympatric *F. cf. longispina* in that the palp of *F. ambigua* has a narrower, more curved embolus, and with the RTA about 3/4 the length of that of *F. cf. longispina*. These two differ from similar Central American species, except true *F. longispina*, by lacking a sharply pointed sclerotized lamella accompanying the embolus near its tip. In addition, the male face of *F. ambigua* has an unbroken white clypeal band (Fig. 1), while in *F. cf. longispina*, the clypeal band is missing medially from the middle of each AME inward. Males of *F. longispina* are like *F. cf. longispina* in having a less curved embolus and more elongate RTA, but like *F. ambigua* in having an unbroken white clypeal band.

Females of *F. ambigua* have the anterior ridged part of the epigyne equal to or longer than the atrial opening. The posterior pocket is incorporated into the floor of the atrium (Fig. 10) and reaches a little past and between the copulatory openings. In *F. cf. longispina*, the anterior ridged area is shorter than the atrial opening (which is larger than in *F. ambigua*), and the posterior pocket extends very near the anterior end of the atrium, corresponding to the longer RTA of the male. The carapace color pattern of females also differs: *F. ambigua* usually has a distinct, narrow, white submarginal band on each side with roughly parallel margins (Fig. 4), whereas in *F. cf. longispina*, the submarginal band is indistinct with a noticeably jagged lower edge. The female of *F. longispina* is not described, at least not under this name, but *Phiale laticava* (F.O.P.-Cambridge 1901) and *Phiale mediocava* (F.O.P.-Cambridge 1901) are apparently related species from Guatemala described only from females.

Description.—*Male* (n = 5): BL 5.05 (6.07) 8.10, CL 2.55 (3.06) 3.85, EFL 1.35 (1.55) 1.90, CW 1.90 (2.26) 3.00, AEW 1.60 (1.88) 2.30, PEW 1.60 (1.88) 2.30, CH 1.20 (1.37) 1.80. Carapace integument black (cephalic) and orange (thoracic), mostly densely covered with elongate black setae adpressed to integument, with most erect setae around eyes. White elongate scale-like setae (scales) make broad marginal band on each side (submarginal at posterolateral corners), broad median stripe from posterior edge of carapace to middle of eye field that comes to sharp point, transverse anterior ocular band just behind AER and complete clypeal band contiguous with marginal bands (Figs. 1, 2). Rust red scales surround AME, ALE, and PME, are dorsal to PLE, and edge AOB and point of median stripe. Chelicerae reddish brown with dense cover of elongate white setae (that also form an elongate fringe on clypeus); promargin with two contiguous teeth, of which outer is larger and about same size as single opposing retromarginal tooth. Endites and labium reddish brown; sternum yellow.

Dorsally palp covered with elongate white setae on femur and patella, elongate black setae on tibia and cymbium. RTA moderately long, bent outward near base, straight or slightly angled ventrally in lateral view, with small inner concavity at tip (Figs. 7, 8). Embolus arising in distal prolateral position, appearing as elongated hook that starts toward the prolateral side before turning back to oblique embolar groove on cymbium angled toward retrolateral side (Fig. 6). Bulb broad with proximal retrolateral lobe that varies in shape, size, and amount of indentation separating lobe from rest of bulb. Legs yellow but extensively marked with black, and femur and tibia I may be mostly black. Leg I ventral macrosetae: metatarsus with two pair (distal, subproximal), tibia with three pair (distal, medial, subproximal). Leg formula I-IV-III-II. Abdomen dorsally with two broad paramedial black stripes, broad median white stripe, and lateral white stripes, respectively covered with same types of black and white setae as carapace. White stripes meet in front to form short basal band. Sides and venter gray, separated by narrow white stripes, and venter sparsely speckled with black.

Female (n = 5): BL 6.00 (7.08) 8.10, CL 2.60 (3.09) 3.40, EFL 1.40 (1.59) 1.70, CW 1.95 (2.29) 2.50, AEW 1.65 (1.92) 2.10, PEW 1.60 (1.91) 2.10, CH 1.30 (1.45) 1.70. Carapace integument reddish brown (cephalic) and orange (thoracic), with median white stripe like male, but narrow white submarginal bands. Dark thoracic areas covered with black setae, but dorsal eye field covered with clear iridescent scales. White submarginal cheek bands, not contiguous with submarginal lateral bands, occur under ALE. Clypeus with long white fringe, but no band. Chelicerae, endites, labium reddish brown; sternum yellow. Cheliceral teeth like male. Palps yellow with dorsal black basal patches on patella, tibia, and tarsus. Legs with less black than males, tending to form distal rings on segments and dorsal femoral stripes, especially femora III and IV; macrosetae as in male. Leg formula IV-III-I-II. Abdomen generally like male dorsally, except dark stripes brown with white speckles, white stripes with brown speckles, and median stripe covered with tan scales; faint chevrons usually present in posterior half of median stripe. Sides dark gray and striated, venter pale with numerous dark gray speckles. Spinnerets brown dorsally, yellowish brown ventrally. Epigyne a low mound with series of parallel transverse ridges along the anterior face, posteriorly with large kidney-shaped atrium that measured along midline is on average slightly shorter than anterior part. Inside atrium is pair of copulatory openings, one each side, that have a rim on posterior edges. Posterior pocket heavily sclerotized, aligned medially, elongate to about half way between the copulatory openings and anterior end of atrium, and has circular opening under (in ventral view) edge of atrium where latter is indented. Copulatory ducts extend posteriorly from anterolateral copulatory openings inside the deep atrium, become slightly sinuous, and lead to small spermathecae located near the posterior edge of the atrium; fertilization ducts emerge from the anterior face of the spermathecae, curving dorsally to the sides. With exception of one Texas male, females noticeably larger than males.

Biology.—In almost all cases with known habitat data, specimens were taken from sunny open dense patches of grass or herbaceous plants with a few small bushes or tree saplings intermixed. This is true in all locations reported here. The spiders were primarily on the grass and herbs, although of the Florida records, one male was taken on a sapling persimmon tree, and a female was taken on a mango tree, about 100 m from a canal that had mixed vegetation along its banks. Habitats included open patches of vegetation in woodland and along trails, as well as vegetation along bodies of water such as fresh water ponds and near saltwater shoreline.

Introduction history.—The first two Florida records (Dixon and Coile 1999, 2000) were from Manatee Co. in the mid-western part of the peninsula. A more recent record (Dixon and Anderson 2010) was from Miami-Dade Co. across the state in the extreme southeast, which might represent either a separate introduction or indicate that the species is now widespread in south Florida. As this paper was being submitted, a new record from this part of the state was found at Dania Beach in Broward Co. Other recent records were the result of surveys in southern Pinellas and Hillsborough counties that were instigated by a photograph of a penultimate male specimen sent to GBE by Lyn Atherton of Tierra Verde. Specimens collected at Tierra Verde and at Ft. DeSoto Park in Pinellas Co., and at Ruskin in Hillsborough Co., along with the Broward Co. specimen, represent new county records for the state. Some of the juveniles from these collections were reared to maturity.

Texas records from Cameron and Hidalgo counties as early as 1981 were found in the TAMUIC collection. Photographic records of a male from Hidalgo Co., Estero Lano Grande State Park, were made by Dick Walton on 21 November 2011, and an apparent penultimate male (based on its distinctive color pattern) was photographed by Terry Fuller in Cameron Co., San Benito, on 28 December 2011 (forwarded by Dick Walton). Although these are the first reports of this species from south Texas, it appears that the species has been established here for over three decades.

Introduction pathway.—Since this species is an inhabitant of moderately low herbaceous vegetation and small shrubs, it is possible that it could have been introduced through the ornamental or florist industries, either with plants intended as yard ornamentals, or with cut flower shipments. There is considerable cut flower traffic from Colombia through the port of Miami (T. Skarlinsky personal communication 2011). Also, *F. ambigua* tends to be found near aquatic environments, and the first Florida record was on Chinese water spinach, *Ipomoea aquatica* Forsskal, an aquatic vine that has become established in Hillsborough, Manatee, and Pinellas counties (Wunderlin and Hansen 2011). This invasive plant is on the USA federal noxious weed list and the Florida prohibited aquatic plant list. Smuggling of this edible plant could have contributed to the presence of *F. ambigua* in Florida. How it became established in Texas is open to speculation.

DISCUSSION

Available evidence suggests that this species is introduced into North America. Despite intensive collecting in the state by several arachnologists throughout much of the 20th century, no previous records were found in Florida, indicating this is a recent introduction. No freyine jumping spiders are known to be native to the Caribbean area except for two species of *Eustiromastix* from St. Vincent in the Lesser Antilles

(Peckham & Peckham 1893), so it seems unlikely that *F. ambigua* naturally dispersed by that route. Texas might be considered more plausible to be in the native range of *F. ambigua* due to its continuous continental connection with the Neotropics. However, the presence of closely related species in Central America, such as *Freya bifurcata* and *Freya longispina*, which are thought to be ecological equivalents, appears to prevent establishment by dispersal northward of *F. ambigua* from northern South America.

Miscellaneous records from various museums have extended the known natural range of *F. ambigua* in South America. Brazil and French Guiana are added to Colombia, Suriname, Trinidad and Tobago, and Venezuela as countries that contain native populations of *F. ambigua*. Thus, the species appears to be widespread in northern South America. It would be predicted to occur at least in Guyana in addition to the countries presently recorded.

Significance.—As a predator, *F. ambigua* is not considered a pest of agricultural significance. However, its potential role as an invasive of natural ecosystems is unknown. It appears that it can occur in high densities in shoreline habitats, both in coastal habitats associated with saltwater and around fresh water ponds. Its potential effect on native salticids that occur in the same habitats, such as the common *Marpissa pikei* (Peckham & Peckham 1888) or the uncommon *Paramaevia hobbsae* (Barnes 1955), remains to be assessed. It is thought at this time that *F. ambigua* will not be able to migrate northward into more temperate climates, and that whatever environmental effects it does have will be restricted to the subtropical zones of south Florida and south Texas.

ACKNOWLEDGMENTS

Lyn and Brooks Atherton kindly hosted GBE in Tierra Verde for three days during November 2011 and April 2012 to survey sites in Pinellas and Hillsborough counties for *F. ambigua*. Jack Koerner (John Koerner Photography, Old Town, Florida, www.macrophotopro.com) photographed a male and a female from the Tierra Verde site (Figs. 1–4). Dick Walton (Richard K. Walton Natural History Services, Concord, Massachusetts, www.rkwalton.com) photographed a Texas male and provided other information on the photographs of Texas specimens. Terry Fuller granted permission to use his photo data. Darci Battesti (IBSP), Gonzalo Giribet and Laura Liebensperger (MCZ), Alexandre Bonaldo (MPEG), Ambros Hänggi (NMB), Edward Riley and Allen Dean (TAMUIC), Jonathan Coddington (USNM), and Jason Dunlop (ZMB) loaned specimens from their respective institutions. Also, Laura Leibensperger photographed the Guatemala specimen of *Menemerus fannae*, and Allen Dean gifted the FSCA with a pair of Texas specimens of *F. ambigua*. Patti Anderson (DPI) and Tom Skarlinsky (USDA/APHIS/PPQ) provided information on introduced plants and cut flower imports in Florida, respectively. Our thanks are given to all for their assistance. This is Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Entomology Contribution #1207.

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Revision of *Bagheera* (Araneae: Salticidae: Dendryphantinae)

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Abstract. The genus *Bagheera* Peckham & Peckham 1896 is revised. Joining *B. kiplingi* Peckham & Peckham 1896 and *B. prosper* (Peckham & Peckham 1901), two new species are described, *B. motagua* sp. nov. from Guatemala and *B. laselva* sp. nov. from Costa Rica. Both sexes of these new species are described and illustrated. Additional illustrations of male palps, epigynes, male chelicerae and habitus of *B. kiplingi* and *B. prosper* are included for comparison, and the females of these two species are formally described for the first time.

Keywords: Jumping spider, new species, systematics, taxonomy, vegetarian spider

The genus *Bagheera* was proposed by Peckham & Peckham (1896) to include *Bagheera kiplingi* Peckham & Peckham 1896 from Guatemala. The genus remained monotypic for a century until the transfer of *Dendryphantes prosper* Peckham & Peckham 1901 by Maddison (1996), resulting in the current list of two species (Platnick 2012).

According to Maddison (1996) and corroborated by an unpublished phylogenetic study with molecular data by GRSR, *Bagheera* may be related to the genera *Gastromicans* Mello-Leitão 1917 and *Messua* Peckham & Peckham 1896 (at least to the *limbata* group of *Messua*), with which it shares a similar folding of the dendryphantine embolus (the embolus arises at the distal end of the tegulum and curls clockwise, as in Fig. 23).

Bagheera kiplingi, the type species of the genus, has recently received attention as the first known spider reported to feed mostly on vegetal sources (Meehan et al. 2009). Although *B. prosper* seems to have the standard predatory behavior, nothing is known about feeding strategies of the new species herein described. The study of feeding strategies in such species could help explain how at least one species of the group evolved towards herbivory.

METHODS

Specimens examined are deposited in the Florida State Collection of Arthropods (FSCA), American Museum of Natural History (AMNH), Instituto Nacional de Biodiversidad (INBio), Instituto Butantan (IBSP), Museum of Comparative Zoology (MCZ) and Texas A&M University Insect Collection (TAMUIC). Numerous specimens were collected as part of the Arthropods of La Selva (ALAS) Project. Some records have two associated codes, an ALAS Project collection code and an INBio accession code. In these cases, the initials of the specific collector are incorporated into the ALAS code.

Habitus and chelicera of the various species were illustrated with camera lucida by holding entire specimens with pins on a foam-covered dish under a stereomiscroscope. Left male palps were dissected and illustrated in high magnification in ventral and retrolateral views. In some cases, curling emboli were illustrated in prolateral view. The leg of *B. motagua* was also illustrated after dissection. External epigynal plates were illustrated still attached to female abdomens. Internal structures were dissected, immersed in clove oil, cleared and illustrated in dorsal view. Epigynes of *B. kiplingi* and *B. prosper* were redrawn, based on previous illustrations by Maddison (1996). Measurements are given in millimeters.

TAXONOMY

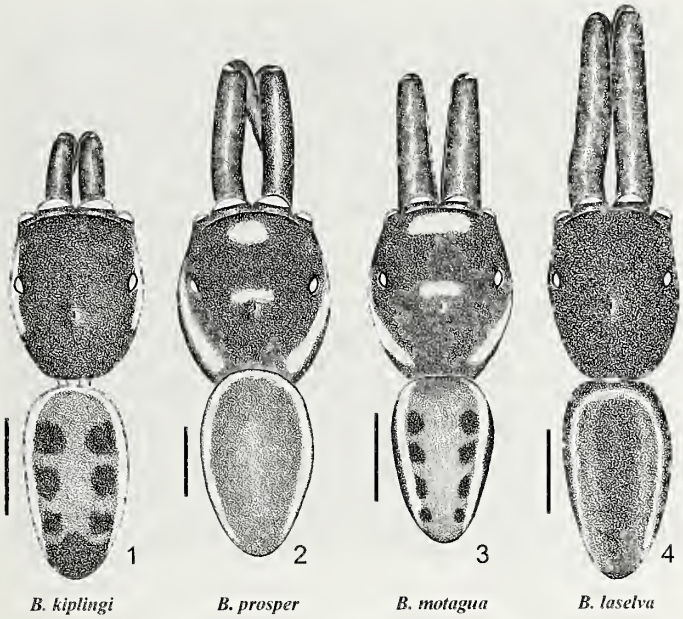
Bagheera Peckham & Peckham 1896

Bagheera Peckham & Peckham 1896 (Type species by monotypy: *Bagheera kiplingi* Peckham & Peckham 1896); Platnick 2012.

Etymology.—The genus was named for the black panther in Rudyard Kipling's The Jungle Book, and the type species was named after the author.

Diagnosis.—*Bagheera* males have elongate, horizontal, parallel chelicerae (Figs. 1–4, 15). The forward projection of the chelicerae is the primary synapomorphy of the genus. The retromarginal single tooth is located near the base of the chelicera (Figs. 5–8). Distally on the basal segment (paturon), near the articulation with the fang, there is a retrolateral cheliceral apophysis that is not a true tooth (Maddison 1996) (Figs. 5, 6, 8). *Bagheera* species have a typical proximal set of two promarginal teeth and one retromarginal tooth, in addition to this cheliceral apophysis.

The palp of *B. laselva* is much like that of *Messua limbata* (Banks 1898). This seems to be the plesiomorphic form of embolus for the clade that includes *Bagheera*, *Messua* and *Gastromicans* and does not indicate any closer relationship within the group. The cheliceral apophysis, however, seems to be a synapomorphy of *Bagheera*, but there is ambiguity between its presence and the shift of the embolus toward the prolateral side. If the presence of the cheliceral apophysis is indeed a synapomorphy of the genus, it would have been lost at least in one species (*B. motagua*). If *B. motagua* were the sister of the other three species (which do have cheliceral apophyses), we would have the embolus shift evolving independently twice (in *B. kiplingi* + *B. prosper* and in *B. motagua*). Moreover, male ornaments on the carapace suggest a close relationship between *B. prosper* and *B. motagua* that is not corroborated by any other feature (Figs. 2, 3). Based on the characters and taxa we have so far, it is not possible to trace character evolution or clearly reconstruct the phylogeny within the genus. This scenario is expected to get a better resolution when other taxa are discovered.



Figures 1–4.—*Bagheera* spp., male body, dorsal view; 1. *B. kiplingi*; 2. *B. prosper*; 3. *B. motagua* sp. nov.; 4. *B. laselva* sp. nov. Scale = 1.0 mm.

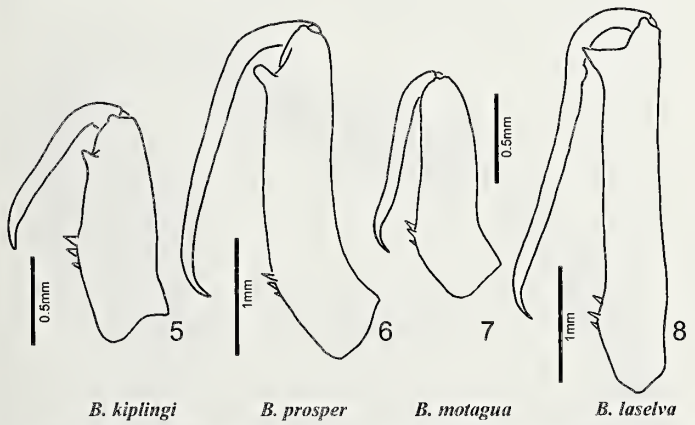
List of species:

Bagheera kiplingi Peckham & Peckham 1896 (type species)
Bagheera laselva sp. nov.
Bagheera motagua sp. nov.
Bagheera prosper (Peckham & Peckham 1901)

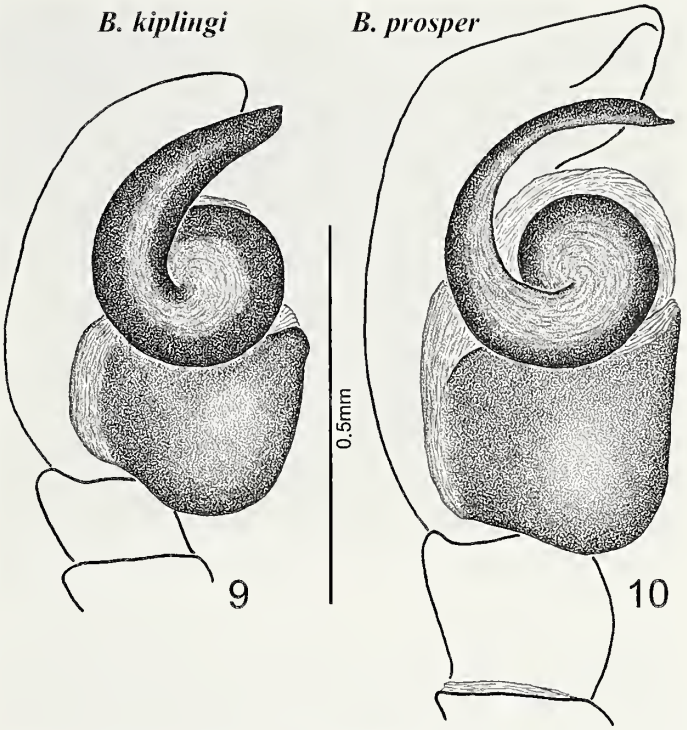
Bagheera kiplingi Peckham & Peckham 1896
Figs. 1, 5, 9, 11, 12

Bagheera kiplingi Peckham & Peckham 1896:88, pl. 7, fig. 1 (Male holotype from Eastern Guatemala, deposited in MCZ, examined); F.O.P.-Cambridge 1901:298, pl. 29, fig. 5; Maddison 1996:335, fig. 71, 80–83; Platnick 2012.

Diagnosis.—Very similar to *B. prosper* by the elongate, laminar embolus relocated on the prolateral side of the tegulum, but can be distinguished from that species by having both edges of the embolus sclerotized (Fig. 9).

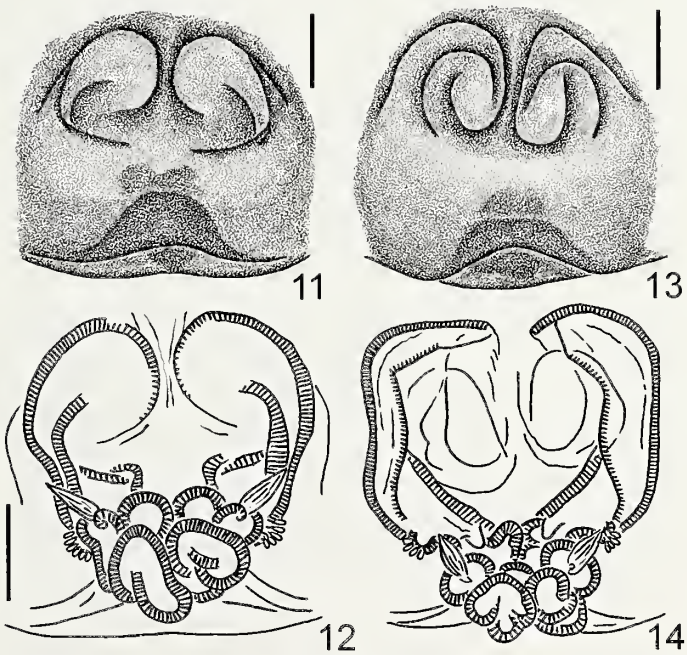


Figures 5–8.—*Bagheera* spp, male left chelicera, retrolateral view; 5. *B. kiplingi*; 6. *B. prosper*; 7. *B. motagua* sp. nov.; 8. *B. laselva* sp. nov.



Figures 9, 10.—*Bagheera* spp, male left palp, prolateral view; 9. *B. kiplingi*; 10. *B. prosper*.

Male.—Male body in dorsal view (Fig. 1) and the male chelicera (Fig. 5) are illustrated here for comparison. For description and further diagnostic illustration, see F.O.P.-Cambridge (1901:298, pl. 29, fig. 5) and Maddison (1996: fig. 81).



Figures 11–14.—*Bagheera* spp, epigyne; 11. *B. kiplingi*, ventral view; 12. *B. kiplingi*, dorsal view, cleared; 13. *B. prosper*, ventral view; 14. *B. prosper*, dorsal view, cleared. Modified from Maddison 1996.

Figure 15.—*Bagheera prosper* live male from Texas, USA. Photo: J.H. Pete Carmichael.

Figure 16.—*Bagheera prosper* live female from Oklahoma, U.S.A. Photo: V. Bugh.

Description.—Female. Total length: 6.15. Carapace reddish dark brown with white scales, 2.50 long, 1.65 wide and 1.05 high. Cephalic region darker, covered with translucent (iridescent?) shiny scales. Length of ocular quadrangle: 1.20. Width of anterior eye row: 1.35, posterior: 1.50. Chelicera reddish brown. Palp, endite, labium and sternum light orange. Legs 142=3, I reddish brown with ventroprolateral region of femur darker, II–IV light orange. Length of femur I: 1.55, II: 1.20, III: 1.12, IV: 1.30; patella + tibia I: 1.75, II: 1.30, III: 1.25, IV: 1.62; metatarsus + tarsus I: 1.25, II: 1.00, III: 1.12, IV: 1.22. Abdomen light, dorsally with a pair of long longitudinal dark brown stripes; posterior half darker, separated from anterior half by light lateral marks. Epigyne with large copulatory openings; flower-shaped glands on the external inner end of the copulatory duct heads (the duct section immediately adjacent to the copulatory duct openings); ducts extend posteriorly and fold on themselves several times, turning into unmodified, poorly developed spermathecae, from which fertilization ducts emerge (Figs. 11, 12). Spinnerets yellow.

Distribution.—Belize, Costa Rica, Guatemala and Mexico (Meehan et al. 2009; Platnick 2012; present study).

Additional material examined.—BELIZE: *Cayo*: Never Delay, 17.316667°N, 88.75°W, August 1959, 1♀ (N.L.H. Krauss, AMNH); GUATEMALA: *Petén*: Parque Natural Ixpanpajul, 16–20 October 2005, 1♂ (G.B. Edwards, FSCA); MEXICO: *Oaxaca*: Puerto Escondido, 15 July 1985, 1♂ (J. Woolley & G. Zolnerowich, TAMUIC); Temescal (5 mi E), 14 June 1964, 4♂ (D.H. Janzen, AMNH); same data, with *Pseudomyrmex ferruginea*, 1♂, 1♀ (D.H. Janzen, FSCA); same location, 1♀, predator of *P. ferruginea*, 13 April–25 May 1964, 3♀, 3 juv (D.H. Janzen, AMNH); *Tamaulipas*: Tampico, 1942, 1♂ (G. Elaboard, AMNH).

Bagheera prosper (Peckham & Peckham, 1901)
Figs. 2, 6, 10, 13–16

Dendryphantes prosper Peckham & Peckham 1901:314, pl. 27, fig. 5 (Two male syntypes from San Antonio, Texas, USA, deposited in MCZ, examined); Peckham & Peckham 1909:475, 477.

Metaphidippus maxillosus F.O.P.-Cambridge, 1901:265, pl. 23, fig. 14 [Male holotype from Orizaba, Mexico, H.H. Smith, deposited in the Godman & Salvin collection (British Museum of Natural History?), not examined]; synonymized by Peckham & Peckham 1909:477.

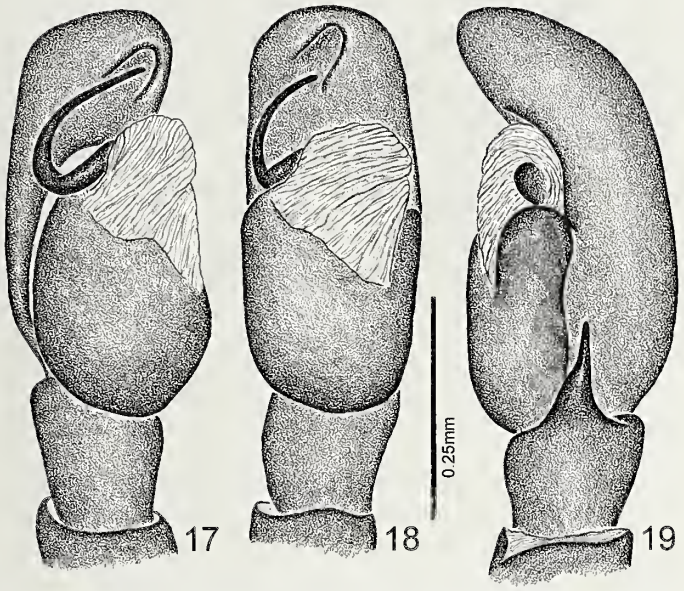
Metaphidippus prosper: Prószyński 1971:434.

Bagheera prosper: Maddison 1996:233, fig. 84–85, 99; Platnick 2012.

Diagnosis.—Very similar to *B. kiplingi* by the elongate, laminar embolus relocated on prolateral side of the tegulum, but can be distinguished from that species by having only the external edge of the embolus sclerotized in the male palp (Fig. 10).

Male.—Males of this species have chelicerae proventrally covered by small tubercles. The male cheliceral length varies considerably, as in *B. laselva*. It is also the largest of the four species (Fig. 1–4). The male body in dorsal view (Fig. 2) and the male chelicera (Fig. 6) are illustrated here for comparison. For description and further diagnostic illustration, see Peckham & Peckham (1901:314, pl. 27, fig. 5) and Maddison (1996, fig. 84).

Description.—Female. Total length: 6.10. Carapace reddish dark brown with white scales, 3.00 long, 1.40 wide and 1.32 high. Cephalic region covered with iridescent scales. Length of ocular quadrangle: 1.50. Width of anterior eye row: 1.90, posterior: 2.10. Clypeus covered with many white scales. Chelicera reddish dark brown. Palp orange. Endite, labium sternum light brown. Legs 1423, I light brown with a dark stripe along the ventroprolateral face of femur (as in *B. kiplingi*) extending to patella and tibia, and dark brown rings distally on patella and subdistally on tibia; metatarsus and tarsus orange; II–IV orange, with same markings present in I and extra dark brown ring on distal femora. Length of femur I: 1.85, II: 1.55, III: 1.50, IV: 1.80; patella + tibia I: 2.45, II: 1.82, III: 1.65, IV: 2.15; metatarsus + tarsus I: 1.62, II: 1.45, III: 1.50, IV: 1.65. Abdomen light, dorsally with five pairs of



Figures 17–19.—*Bagheera motagua* sp. nov., left male palp; 17. proventral view; 18. ventral; 19. retrolateral.

dark brown, irregular spots (but symmetrical within each pair), the posterior three pairs sometimes fused forming chevrons or W's; on inner side of each dark brown spot is a patch of white scales (Fig. 16); ventrally with a median, longitudinal dark brown stripe. Epigyne with large copulatory openings; flower-shaped glands on the external inner end of the copulatory duct heads; ducts extend posteriorly and fold on themselves several times, turning into unmodified, poorly developed spermathecae, from which fertilization ducts emerge (Figs. 13, 14). Spinnerets light brown.

Distribution.—USA and Mexico (Platnick 2012). The Arkansas and Oklahoma records are the first for those states.

Additional material examined.—MEXICO: *San Luis Potosi*: El Salto (W Antiguo Morelos), 20 June 1953, 1♂ (P. & C. Vaurie, AMNH); *Veracruz*: Mantla, 1 July 1946, 2♀, 2juv (H. Wagner, AMNH); USA: *Arkansas*: Pulaski Co., Little Rock, Riverfront Park along Arkansas River, 34.749256°N, 92.268317°W, 8 June 2012, 1♂ (R.K. Walton, FSCA); *Oklahoma*: Marshall Co., Willis, University of Oklahoma Biological Station, 26 June 2012, 3♂, 1♀, 2 juv (P.K. Morton, FSCA); *Texas*: Atascosa Co., San Antonio (30 mi S), 20 August 1935, 1♂ (S. Mulaik, AMNH); Burnet Co., junc. Hwys 29 & P4 at bridge, stream edge vegetation, 13 July 1993, 1♀ reared (G.B. Edwards & P.D. Barron, FSCA); Comal Co., New Braunfels, on tree, 12 April 1936, 1♂, 1♀ (S. Mulaik, AMNH); Hays Co., San Marcos, 31 March 1936, 1♂, 1 juv (AMNH); Hunt Co., Lake Tawakoni, on leaf, 22 September 2007, 1♂ (S.R. Dean, TAMUIC); Kimble Co., Llano River, S. London on Hwy 385, 14 March 1982, 1♂ (J.C. Cokendolpher, FSCA); same county, Junction, 15 October 2001, 1♂ (L.A. Brooks, TAMUIC); Llano Co., nr. Ferguson Power Plant, creek vegetation, oak, cedar, mesquite, 13 July 1993, 1♂, 3♀, 1 juv (G.B. Edwards & P.D. Barron, FSCA); same data, 1♂ (G.B. Edwards & P.D. Barron, FSCA); Llano City Lake Park, lake edge, cedar, elms, oaks etc., 13 July 1993, 2♂ reared (G.B. Edwards & P.D. Barron, FSCA); same county, August 1935, 1♂ (L.I. Davis, AMNH); Runnals Co., Lake Ballinger, lake edge, grasses and herbs, 11 July 1993, 2♂ reared (G.B. Edwards & P.D. Barron, FSCA); same county, junction Colorado, 4♂, 1♀, 1 juv reared (G.B. Edwards & P.D. Barron, FSCA); Starr Co., near Falcon Dam, 20 April 1985, 2♂ (J.B. Woolley 85/004, TAMUIC); same county, Salineño, Lower Rio Grande Valley National Wildlife Refuge, 26.515264°N, 99.115142°W, el. 20m, FIT – riparian mesquite forest, 4 March–3 April 2004, 1♂ (S. & J. Peck, TAMUIC); Tom Green Co., 1974–1980, 2♀ (N.K. & J.L. Fisher, FSCA); Val Verde Co., vegetation, 21 October 1972, 1♂ (J.F. Parrish, FSCA).

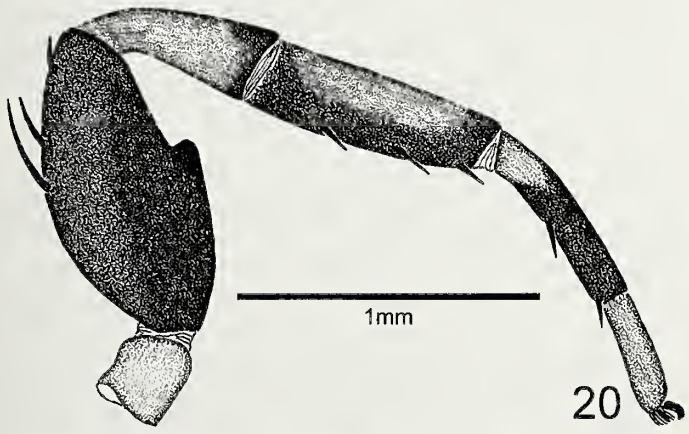
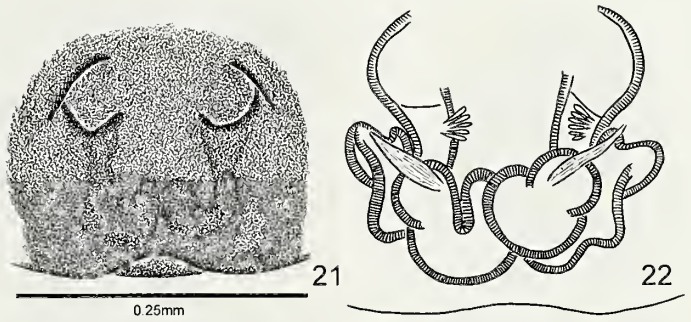


Figure 20.—*Bagheera motagua* sp. nov., left male leg I, prolateral view.



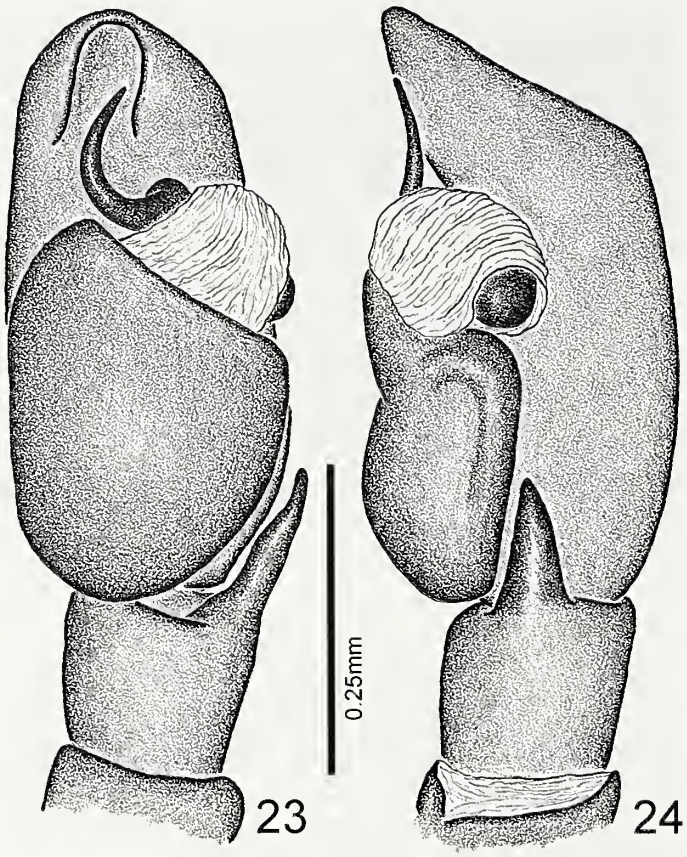
Figures 21, 22.—*Bagheera motagua* sp. nov., female epigyne; 21. ventral view; 22. dorsal view, cleared.

Bagheera motagua sp. nov.
Figs. 3, 7, 17–22

Types.—Male holotype, 1 male and 4 female paratypes, from El Progreso, Guatemala (highway between El Rancho and Cobán, km 190, along Motagua River, elev. 600m), 12 October 2005, G.B. Edwards (FSCA, IBSP).

Etymology.—The epithet is a toponymy in apposition and refers to the river along which the type specimens were found.

Inclusion in *Bagheera*.—Despite males not having the typical distal retrolateral cheliceral apophyses (Fig. 7), the chelicerae are elongate, horizontal and parallel (Fig. 3) and bear small proventral tubercles similar to those of *B. prosper*. The thin embolus is elongate and relocated to the prolateral side of the tegulum, as those of *B. kiplingi* and *B. prosper*. The male carapace has the same pattern of white scales as that found on the carapace of *B. prosper* (Figs. 2, 3), while the abdominal pattern is similar to that of *B. kiplingi* (Figs. 1, 3). In addition,

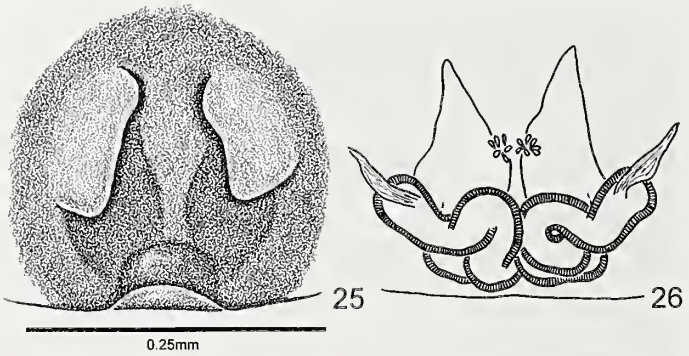


Figures 23, 24.—*Bagheera laselva* sp. nov., left male palp; 23. ventral view; 24. retrolateral.

males have dark marks on proventral femora I and II, similar to those of *B. kiplingi* (Fig. 20).

Diagnosis.—Males can easily be recognized by a ventral projection on femora I (Fig. 20), while females can be distinguished by having small copulatory openings (Fig. 21).

Description.—Male. Total length: 4.25. Carapace dark brown with lateral stripes of white scales, a group of white scales right behind the anterior median eyes and another right in front of fovea (Fig. 3). Rest of carapace covered with iridescent scales, especially cephalic region. Carapace 2.15 long, 1.70 wide and 1.05 high. Length of ocular quadrangle: 1.10. Width of anterior eye row: 1.30, posterior: 1.45. Chelicera dark brown with two promarginal and one retromarginal tooth, all proximal (Fig. 7). Endite, labium and sternum yellow. Palp yellow, with straight and acute retrolateral tibial apophysis (Fig. 19), round cymbium and tegulum, well developed embolic hematodocha and relatively long embolus, arising at distal end of tegulum and curling to prolateral side and back to ventral position, the tip lying in oblique ventral groove (Figs. 17, 18). Legs 1423. Leg I yellow, with dark brown marks on following areas: proventral femur, distal prolateral patella, ventral tibia and ring on distal half of metatarsus (Fig. 20). Ventral projection well developed on ventral femur I (Fig. 20) and poorly developed on femur II. Legs II–IV yellow, II with dark brown marks on proventral femur. Length of femur I: 1.45, II: 1.05, III: 1.05, IV: 1.20; patella + tibia I: 2.05, II: 1.30, III: 1.10, IV: 1.45; metatarsus + tarsus I: 1.40, II: 1.00, III: 1.05, IV: 1.10. Abdomen yellow



Figures 25, 26.—*Bagheera laselva* sp. nov., female epigyne; 25. ventral view; 26. dorsal view, cleared.

dorsally with four pairs of dark brown spots and lateral stripes of white scales (Fig. 3); area among pairs of dark spots covered with clear iridescent scales; ventrally yellow. Spinnerets yellow.

Female.—Total length: 4.05. Carapace dark brown with sparse white scales, 1.65 long, 1.30 wide and 0.75 high. Length of ocular quadrangle: 1.40. Width of anterior eye row: 1.10, posterior: 1.22. Chelicera dark brown. Palp and endite yellow. Labium dark brown and sternum yellow. Legs 4123, yellow, with no markings. Length of femur I: 0.90, II: 0.75, III: 0.75, IV: 1.05; patella + tibia I: 1.10, II: 0.85, III: 0.80, IV: 1.10; metatarsus + tarsus I: 0.77, II: 0.70, III: 0.72, IV: 0.90. Abdomen light with four well developed pairs of dark brown spots, the fourth medially fused, and narrow transverse dark stripe right in front of spinnerets; iridescent scales on longitudinal stripe among dark spots and spots separated by narrow transverse bands of white scales. Epigyne with small copulatory openings; flower-shaped glands on dorsum of copulatory duct heads; ducts extend posteriorly and immediately to lateral sides, fold dorsally back to middle and enter poorly developed spermathecae, from which fertilization ducts emerge (Figs. 21, 22). Spinnerets yellow.

Remarks.—Male-female matching established based on co-occurrence in type locality.

Biological notes.—The specimens were collected by beating in a xeric area dominated by *Acacia* sp. and other desert shrubs. The habitat suggests that this species has a diet similar to *B. kiplingi*, or if not, it might provide comparative behavioral data on the evolution of different dietary choices under similar environmental conditions.

Bagheera laselva sp. nov.
Figs. 4, 8, 23–26

Types.—Male holotype from Turrialba, Costa Rica, 16 August 1963, W. Peck (FSCA); 6 male paratypes from Estación Biológica La Selva, Heredia, Costa Rica, 10.433333°N, 84.016667°W, January 1997, INBio-OET (FSCA, IBSP); 9 female paratypes from Estación Biológica La Selva, Heredia, Costa Rica, November 1996 (FSCA, IBSP).

Etymology.—The epithet is a toponomy in apposition and refers to the Estación Biológica La Selva, Costa Rica, from where most of the type specimens were collected.

Diagnosis.—This species has a well developed distal retrolateral male cheliceral apophysis (Fig. 8), similar to that

present in *B. kiplingi* and *B. prosper*. However, this is the only known species of the genus whose embolus is short and not remarkably relocated on the prolateral side (Fig. 23). Females can be recognized by the large copulatory openings at the inner border of large light areas (Fig. 25).

Description.—Male. Total length: 4.40. Carapace dark brown with sparse white scales, 2.10 long, 1.45 wide and 0.97 high (Fig. 4). Length of eye quadrangle: 1.07. Width of anterior eye row: 1.35, posterior: 1.35. Chelicera dark brown with two promarginal and one retromarginal tooth, all proximal, and a well developed distal, retrolateral, cheliceral apophysis (Fig. 8). Length of the proximal article of chelicera: 2.65. Endite, labium and sternum dark brown. Palp dark brown, with straight and triangular retrolateral tibial apophysis, round tegulum, well developed embolic hematodocha and short, curved embolus at the distal end of the tegulum (Figs. 23, 24). Legs 1423 dark brown, with coxae and trochanters yellow; posterior half of coxa IV dark brown. Length of femur I: 1.27, II: 1.10, III: 1.10, IV: 1.35; patella + tibia I: 1.95, II: 1.40, III: 1.20, IV: 1.60; metatarsus + tarsus I: 1.52, II: 1.15, III: 1.15, IV: 1.35. Abdomen dorsally light brown with a median longitudinal dark brown stripe covered with iridescent scales and lateral stripes of white scales (Fig. 4); ventrally dark brown. Spinnerets dark brown.

Female.—Total length: 5.40. Carapace light brown with sparse white scales, 1.95 long, 1.45 wide and 0.90 high. Length of the eye quadrangle: 1.05. Width of anterior eye row: 1.25, posterior: 1.35. Chelicera light brown. Palp yellow with a dark brown mark on proximal dorsal tibia and tarsus. Endite, labium and sternum light brown. Legs 4123, yellow. Leg I with dark brown rings on subdistal femur, distal half of patella and distal half of tibia; II with dark brown mark on distal prolateral patella; III with dark brown mark on distal retrolateral tibia; IV with dark brown marks on the prolateral and retrolateral faces on subdistal femur and distal patella, and black rings on distal tibia and metatarsus. Length of femur I: 1.10, II: 1.00, III: 0.97, IV: 1.25; patella + tibia I: 1.45, II: 1.22, III: 1.07, IV: 1.50; metatarsus + tarsus I: 1.07, II: 0.97, III: 1.10, IV: 1.22. Abdomen dorsally light with four well developed pairs of dark brown spots and a narrow transverse dark brown band right in front of the spinnerets; iridescent scales on the spots; front three pairs of dark spots with small tufts of white scales on their medial borders; third and fourth spot pairs separated by thin band of white scales; ventrally with longitudinal light brown stripe. Epigyne with large copulatory openings, with flower-shaped glands at the inner border of large duct heads; copulatory ducts extend toward the posterior border, then to the middle, fold dorsally and then to the lateral sides, entering poorly developed spermathecae, from which fertilization ducts emerge (Figs. 25, 26). Spinnerets dark brown.

Remarks.—Male-female matching established based on co-occurrence in type locality. Some males have smaller chelicerae, of about the same length as the carapace. These shorter chelicerae are also straighter and do not have the proximal arch.

Biological notes.—The La Selva specimens were collected at 50–150m elevation, by sweeping tall and often dense grass in early successional stages where the forest had been cleared. Additional specimens were collected nearby on Mt. Barva at 450–550m.

Additional Material Examined.—COSTA RICA: Heredia: 10 km SE La Virgen, 10.333333°N, 84.083333°W, 450–550m, primary succession, 8–22 April 2003, 1♀, 1 juv (G.S. Bodner & G.B. Edwards, GBE4.10.03SW1Cl, FSCA); same data, 2♂, 6♀, 3 juv (G.S. Bodner & G.B. Edwards, FSCA); Estación Biológica La Selva, 10.433333°N, 84.016667°W, 50–150m, September 1996: 1♀ (G.B. Edwards, G.S. Bodner, D. Brenes, R. Vargas, M. Paniagua, N. Oconitrillo, AGBE96-1 INBIOCR1002734421, INBio/FSCA); same data for all following records except as noted: 1♂ (AGBE96-1 INBIOCR1002734425); 1♀ (AGBE96-12 INBIOCR1002735165); 1♂, 2♀ (AGBE96-18 INBIOCR1002735081); 1♂ (AGSB03setGO2 INBIOCR1002069504); 1♀ (AGSB03setRE1 INBIOCR1002069562); 1♀ (AGSB06setGO1 INBIOCR1002069651); 1♂, 1♀ (ANOM03setRE1 INBIOCR1002069497); 1♂ (ARVC03 setRE1 INBIOCR1002069498); 1♂ (ASCNOMBA04 INBIOCR1002735619); November 1996: 3♂ (AHBRVCRE02 INBIOCR1002737731); 1♂ (AHCRVCRE01 INBIOCR1002737758); 3♂ (AHCRVCRE02 INBIOCR1002737738); 2♂ (AHCRVCRE03 INBIOCR1002737843); 5♀ (AHCRVCRE03 INBIOCR1002737847); 1♂ (AHCRVCRE04 INBIOCR1002737828); 11♀ (AHCRVCRE04 INBIOCR1002737837); 2♂ (AHCRVCRE05 INBIOCR1002737812); 3♀ (AHCRVCRE06 INBIOCR1002737819); 3♂ (AHCRVCRE06 INBIOCR1002737822); December 1996: 6♀ (AHBRVCBA06 INBIOCR1002738025); 8♂ (AHCRVCBA01 INBIOCR1002737970); 5♀ (AHCRVCBA01 INBIOCR1002737972); 11♂ (AHCRVCBA02 INBIOCR1002737961); 9♀ (AHCRVCBA02 INBIOCR1002737962); 8♂ (AHCRVCBA03 INBIOCR1002737981); 10♂ (AHCRVCBA04 INBIOCR1002738001); 7♀ (AHCRVCBA04 INBIOCR1002738004); 9♀ (AHCRVCBA05 INBIOCR1002737996); 1♂ (AHCRVCGO01 INBIOCR1002737871); 4♀ (AHCRVCGO06 INBIOCR1002737905); 3♂ (AHCRVCGO06 INBIOCR1002737906); January 1997: 1♂ (AHBDBMGO01 INBIOCR1002738482); 1♂ (AHBMPGGO08 INBIOCR1002738436); 1♀ (AHBMPGRE04 INBIOCR1002738587); 1♂ (AHBMPGRE04 INBIOCR1002738593); 1♀ (AHBNOMRE02 INBIOCR1002738352); 2♂ (AHBNOMRE02 INBIOCR1002738357); 1♂ (AHBNOMRE04 INBIOCR1002738371); 2♀ (AHCDBMGO04 INBIOCR1002738224); 1♂ (AHCDBMGO05 INBIOCR1002738238); 6♀ (AHCDBMRE01 INBIOCR1002738243); 4♂ (AHCDBMRE01 INBIOCR1002738254); 4♀ (AHCDBMRE02 INBIOCR1002738260); 1♂ (AHCDBMRE02 INBIOCR1002738271); 3♂ (AHCDBMRE03 INBIOCR1002738289); 1♂ (AHCDBMRE03 INBIOCR1002738295); 2♀ (AHCDBMRE04 INBIOCR1002738300); 1♂ (AHCDBMRE04 INBIOCR1002738320); 2♀ (AHCDBMRE05 INBIOCR1002738330); 1♂ (AHCDBMRE06 INBIOCR1002738338); 1♂ (AHCDBMRE06 INBIOCR1002738343); 1♂ (AHCMPGBA01 INBIOCR1002738687); 2♀ (AHCMPGGO01 INBIOCR1002738621); 1♂ (AHCMPGGO01 INBIOCR1002738622); 1♀ (AHCMPGGO01 INBIOCR1002738655); 1♀ (AHCMPGGO02 INBIOCR1002738640); 2♀ (AHCMPGGO06 INBIOCR1002738671); 1♂ (AHCMPGGO06 INBIOCR1002738676); 6♂ (AHCMPGRE01 INBIOCR1002738871); 1♀ (AHCMPGRE04 INBIOCR1002738894); 4♂ (AHCMPGRE04 INBIOCR1002738897); 3♀ (AHCMPGRE05 INBIOCR1002738905); 1♂ (AHCMPGRE05 INBIOCR1002738910); 5♀ (AHCMPGRE06 INBIOCR1002738915); 9♂ (AHCMPGRE06 INBIOCR1002738917);

1♂ (AHCNOMGO02 INBIOCR1002738454); 1♀ (AHCNOMGO06 INBIOCR1002738529); 1♀ (AHCNOMRE01 INBIOCR1002738558); 1♂ (AHCNOMRE01 INBIOCR1002738559); 3♀ (AHCNOMRE02 INBIOCR1002738745); 2♂ (AHCNOMRE02 INBIOCR1002738750); 6♂ (AHCNOMRE03 INBIOCR1002738753); 4♀ (AHCNOMRE03 INBIOCR1002738761); 5♀ (AHCNOMRE04 INBIOCR1002738763); 8♂ (AHCNOMRE04 INBIOCR1002738771); 3♂ (AHCNOMRE05 INBIOCR1002738793); 1♂ (AHCNOMRE05 INBIOCR1002738796); 7♀ (AHCNOMRE05 INBIOCR1002738801); 15♀ (AHCNOMRE06 INBIOCR1002738809); 4♂ (AHCNOMRE06 INBIOCR1002738834.

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Does behavioral isolation prevent interspecific mating within a parallel ecotypic wolf spider radiation from the Galápagos?

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Abstract. Behavioral isolation may play an important role in speciation. However, the roles of divergence time and ecological specialization on the evolution of intrinsic barriers to gene flow are poorly understood. On the Galápagos, ecotypic differentiation of *Hogna* Simon 1885 wolf spiders has led to the repeated evolution of morphologically distinct high-elevation and coastal species on Santa Cruz and San Cristóbal. This offers a unique opportunity to investigate the importance of ecological factors and evolutionary history on courtship behavior, but also to explore the opportunity for interspecific gene flow. On San Cristóbal, both high elevation and coastal *Hogna* species clearly showed distinct courtship behavior. This pattern corresponded primarily with variation in male genital organs rather than with ecotypic classification or phylogenetic relationship. Despite low acceptance rates, heterospecific mating was observed, suggesting that potential gene flow within as well as among islands should not be neglected when seeking to understand island radiations.

Keywords: Gene flow, parallel evolution, natural selection, sexual selection

The speciation process necessarily involves the reduction of gene flow between actually or potentially interbreeding populations (Coyne & Orr 2004; Futuyma 2005). If populations diverge in allopatry, spatial isolation serves as an initial isolating barrier (Coyne & Orr 2004). This initial barrier might be re-enforced due to the accumulation of differentially selected traits that reduce interspecific attraction and therefore heterospecific mating; i.e., behavioral/sexual isolation (Andersson 1994; Schluter 2000; Panhuis et al. 2001; Masta & Maddison 2002; Rundle & Nosil 2005). In general, mating traits are predicted to diverge between populations due to mechanisms that are not related to the environment, such as genetic drift and sexual selection (Futuyma 2005). In contrast, in the light of ecological speciation, the evolution of mating traits is predicted to correlate with the environment as a byproduct of natural selection (Boughman 2001; McKinnon & Rundle 2002; Rundle & Nosil 2005) and may as such lead to assortative mating of populations that have undergone similar selection pressures. Hence, scenarios wherein allopatric populations each diverged along a similar selection gradient (i.e., parallel divergence) provide a unique opportunity to test the respective roles of sexual and natural selection in the evolution of behavioral isolation (Boughman 2001; Boughman et al. 2005). Although mating traits most frequently evolve independently among species when populations are isolated, they can also be expected to evolve by speeies interactions when the diverging populations come into secondary contact and suffer reduced hybrid viability (Dobzhansky 1937; Coyne & Orr 1989).

As behavioral isolation is expected to evolve rapidly between incipient species (Del Solar 1966; Gleason & Ritchie 1998) and even faster than intrinsic postzygotic isolation barriers (Coyne & Orr 1989, 1997, 2004; Mendelson 2003), we here focus on the role of behavioral isolation in the radiation of the ground-dwelling wolf spider genus *Hogna* Simon 1885 from the Galápagos (De Busschere et al. 2010, 2012). As male wolf spiders need to persuade females by courting, differences in courting signals are expected to serve as prezygotic behavioral isolating mechanisms (Andersson 1994; Uetz 2000; Rypstra et al. 2009). Moreover, male wolf spider courtship, which may involve different sensory channels such as visual, vibratory and chemical signals, often leads to elaborate species-specific male courtship displays (Miller et al. 1998; Hebets & Uetz 2000), enabling delineation of species boundaries between morphologically indistinguishable species (Den Hollander & Dijkstra 1974; Uetz & Denterlein 1979; Töpfer-Hofmann et al. 2000; Chiarle et al. 2010).

Within-island habitat specialization was demonstrated to lead to morphologically highly similar *Hogna* species in similar habitats on both San Cristóbal and Santa Cruz (De Busschere et al. 2010, 2012) (Fig. 1). *Hogna galapagoensis* Banks 1902 and *H. junco* Baert & Maelfait 2008 are morphologically difficult to distinguish (with the exception of genital traits) and are referred to as "high elevation species" occurring on the top of Santa Cruz and San Cristóbal in the dense pampa vegetation dominated by ferns and sedges (Fig. 1). Similarly, *H. hendrickxi* Baert & Maelfait 2008 and *H. snodgrassi* Banks 1902 are morphologically difficult to distinguish and are referred to as coastal dry species found in the dry supralittoral and arid zone along the coast in sparsely vegetated dunes and open shrub land on Santa Cruz and San Cristóbal (Baert et al. 2008c) (Fig. 1). High-elevation species are characterized by darker eoloration, smaller body size and smaller eyes than eoastal dry species (De Busschere et al. 2012). Contemporary gene flow between these species appears absent, based on allozyme allele frequencies (Baert et al. 2008a) and on spatial isolation of high elevation and coastal dry habitats (Fig. 1). Nevertheless, on Santa Cruz, ecological divergence between *H. galapagoensis* and *H. hendrickxi* has been shown to occur in the face of low levels of gene flow (De Busschere et al. 2010), which is in agreement with the very similar shape of their male copulatory organs (De Busschere et al. 2012). Moreover, based upon Loosveldt (2004) and our sampling campaigns, these *Hogna* species seem to have a similar seasonal life cycle, in which activity is concentrated in the warm wet season from January to May, suggesting no potential role for strong

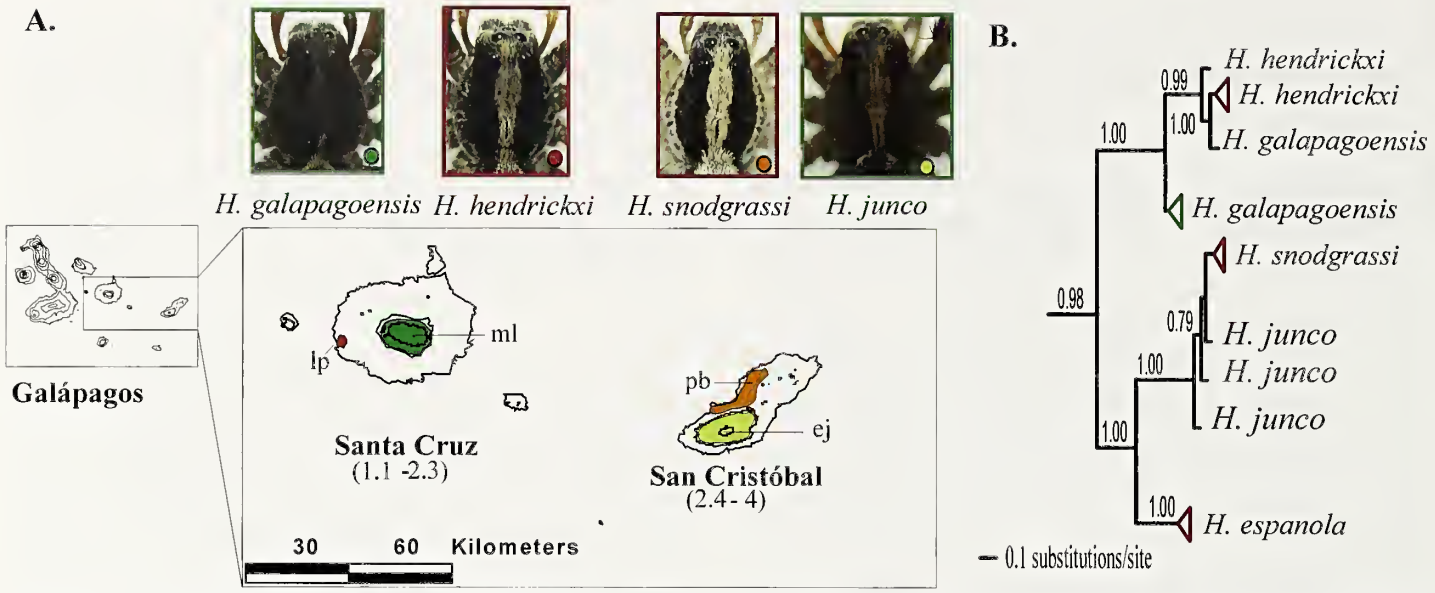

Figure 1.—(A) Geographical distribution (adapted from De Busschere et al. 2010) and (B) COI-28S phylogeny (De Busschere et al., 2010) of high elevation (green) and coastal (red) *Hogna* species on Santa Cruz and San Cristóbal (with exception of *H. espanola*). Node values represent Bayesian posterior probabilities; sampling localities are abbreviated as: Las Palmas (lp), Media luna (ml), Punta Bassa (pb) and Volcán El Junco (ej), and estimated minimum and maximum geological ages (MYA) for each island are in parentheses (D. Geist et al. unpubl . data).

temporal isolation. The latter observation and the signature of historical gene flow demonstrate that gene flow between *H. galapagoensis* and *H. hendrickxi* might still be possible, despite their ancient initial split (~0.8 My) (De Busschere et al. 2010).

This study system allows us to investigate whether strong ecological divergence and/or historical gene flow has led to behavioral isolation by addressing the following research questions: 1) Are there interspecific differences in male courtship behavior, and are these differences related to differences in ecology, or do they correlate with the phylogenetic relationships among the species? 2) Do interspecific differences serve as effective prezygotic isolating barriers? To address these questions, we documented male courtship behavior and performed interspecific mating trials among the four *Hogna* species for which within-island habitat specialization was demonstrated on San Cristóbal and on Santa Cruz.

METHODS

Sampling.—Juvenile and adult *H. galapagoensis* and *H. hendrickxi* from Santa Cruz were sampled at Media Luna (pampa, high elevation) and Las Palmas (coastal), respectively. The species from San Cristóbal, *H. junco* and *H. snodgrassi*, were sampled at Volcán El Junco (pampa, high elevation) and Punta Bassa (coastal) in February 2010, respectively (Fig. 1). Sampling efforts led to a total of 431 specimens with sample sizes ranging from 64 to 187 individuals per species, 52% of which were juveniles. They were housed individually in the quarantine laboratory of the Charles Darwin research station at an average temperature of 20°C and fed ad libitum with two to three wild-caught moths per day (adults) or five fruit flies per day (juveniles). Although many juveniles exuviated in the lab, none of them reached adulthood, suggesting that our laboratory conditions did not adequately mimic the field conditions to induce maturation. Since we could not ensure virginity of the females used in the mating experiments, no reliable quantitative comparisons can be made concerning the degree of inter- and interspecific acceptance rates (see below).

Analysis of species-specific male courtship behavior.—Wild-caught individuals were used in mating trials to describe the species-specific courtship behavior of the four species. The use of wild-caught individuals might confound results due to mating experience, age and mating status of the female. Therefore, we restricted our aim to describing the presence or absence of species-specific male courtship behaviors. Mating trials were performed in a plastic arena (30 × 20 cm) filled with 1 cm of sand. Before each trial, a new filter paper was placed on the sand in order to eliminate signals from previous trials and allowing vibrations. Females were placed in the arena and confronted with 1) a conspecific male, 2) a heterospecific ecotypically similar male (from the other island) or 3) a heterospecific ecotypically dissimilar male (from the same island) after a 5 min period of acclimatization. Trials were observed on average for 20 min. This time frame was chosen based on an initial subset of trials for which we observed that if mating were to occur, it was generally completed within the first 5 min of the experiment. Courtship events were recorded with a HDV camera (SONY HV40 Legria). For each mating trial, the longest recorded complete courtship fragment within those 20 min was used for further analyses. These fragments were chosen if we observed several stages of the mating process starting from the male detecting the female, approaching her and then elaborately courting her until she responded.

A list of five recognizable male courting behaviors was defined and used to score male courtship behavior during the fragment (Table 1; Video 1, online at http://www.bioone.org/doi/suppl/10.1636/K12-49). Females' reaetions were classified as 1) acceptance of the male (i.e. allowing him to mount), 2) aggressive behavior, or 3) no response. For each mating trial, total male courting time (t_{tot}) was assessed, and the absence/presence of male courting behavior was obtained.

Table 1.—Description of male courtship behaviors (see Video 1).

PM	Pedipalp movements: this involves all movements of the male pedipalps and mainly consisted of drumming against the substrate.
FM	Foreleg movements: this involves all movements with the first two pairs of legs and consists of repeatedly raising, waving, tapping, arching and stretching forelegs.
mP	Moderate push-ups: this involves a period of repeated moderate push-ups of the total body invoked by bending the legs.
sP	Strong push-ups: this involves a period of repeated strong push-ups of the total body invoked by strongly bending the legs and leading to jumps.
Po	Poking: repeated poking of the female with the forelegs; forelegs are positioned in front of the male and parallel with the substrate.

First, we tested whether males courted differently; i.e., expressed different courting behaviors to heterospecific females than to conspecific females. This was done for each species by comparing the probability of occurrence of each male courtship behavior toward the females of different species by means of exact Pearson Chi-Square tests (StatXact-5). If no differences were observed in the presence or absence of male courtship behavior with respect to the species of the female, data for heterospecific and conspecific trials were pooled to describe species-specific male courtship. Second, interspecific differences in the probability of expressing a specific courtship behavior among males of the different species were tested with exact Pearson's Chi-Square tests to look for the presence of species-specific courtship behaviors.

Inter- vs. intraspecific courting.—Investigating the potential for reproductive isolation should ideally be based upon heterospecifie and conspeeific choice and no-choice trials using virgin adults. Here, the lack of virgins impedes us from investigating mate preferences directly. However, heterospecific mating trials allowed us to observe whether heterospeeific females elicited male courting behavior and whether females could distinguish and reject heterospecific males. Observations of heterospecific acceptances under laboratory conditions might indicate the presence of weak premating barriers. By means of exact Pearson's Chi-Square tests, we tested whether the probability of male courting differed with respect to female species.

RESULTS

Interspecific comparison in male courtship behavior.—Table 2 gives an overview of the total number of trials performed and the number of trials used in the analysis of interspecific comparisons of male courtship behavior. Given that particular courtship traits were consistently expressed irrespective of the species of the female to which the male was exposed to ($P > 0.17$), male courtship data were pooled across female species. Movements of the pedipalps (PM) were observed in males in all four species (Table 3). For the other courtship traits, large differences were observed among species (Table 3). Courtship of males of both species from San Cristobal can be clearly distinguished, based on some unique male courting behaviors. *Hogna snodgrassi* males often court for extremely long periods (up to 12 min) by combining palpal drumming with strong push-ups (sP) (Table 3). In comparison, *H. junco* males generally court for much shorter periods and combine pedipalp drumming and quick movements toward the female, and if distance is small, males poke the females repeatedly with their forelegs (Po). Differences in courtship between males of the two Santa Cruz species; i.e., *H. galapagoensis* and *H. hendrickxi*, are much less evident, and both species combine palpal drumming, elaborate movements of the forelegs and moderate push-ups while courting. The latter courtship trait was not observed for the two species from San Cristóbal. Although our quantitative measurements of the courtship of both Santa Cruz species were not significantly different (Table 3), some subtle differences were observed, wherein *H. hendrickxi* males tended to make more use of the second pair of forelegs than *H. galapagoensis* males and often moved their pedipalps sidewards while drumming (C. De Busschere pers. observ.). In sum, the species on San Cristóbal, *H. junco* and *H. snodgrassi,* are clearly distinguishable, based upon unique male courtship behaviors.

Table 2.—Sample sizes of total trials and, in parentheses, trials used in courtship analysis.

	Females			
Males	*gala*	*hend*	*snod*	*junc*
gala	37 (12)	9 (4)	–	13 (5)
hend	13 (5)	13 (6)	3 (1)	–
snod	–	4 (1)	16 (9)	6 (0)
junc	11 (9)	–	5 (4)	16 (10)

Inter and intraspecific copulations.—Males apparently did not prefer conspecific females, as the number of courtship events a male displayed was not significantly different when exposed to heterospecific females ($P > 0.45$). Although the acceptanee rate of courting males was in general very low (10%), few heterospecific mating events were observed, and the acceptance rate among species did not differ from random ($X^2 = 2.6$, $P = 0.46$) (Fig. 2). Remarkably, despite clear differences in morphology, *H. galapagoensis* females accepted heterospecific males of *H. hendrickxi* Moreover, *H. galapoensis* females also accepted heterospecific males from the distantly related *H. junco*, which has a distinctively different male courtship (Poking) (Fig. 2).

DISCUSSION

Interspecific differences in male courtship behavior.—The mating trials revealed that the high-elevation species *H. junco* and the coastal species *H. snodgrassi,* both from San Cristóbal, show distinct male courtship behaviors. In contrast, our quantitative analysis based on five male courtship traits did not reveal any significant differences between *H. hendrickxi* and *H. galapagoensis* on Santa Cruz. The lack of difference between these species is in strong concordance with earlier studies (Baert et al. 2008b; De Busschere et al. 2012) that noted almost identical male genital traits for *H. hendrickxi* and *H. galapagoensis,* which clearly differ from those of the San Cristóbal species. Furthermore, De Busschere et al. (2012) observed clear interspecific differences in male genital traits between *H. junco* and *H. snodgrassi.* Hence, the variation in male courtship behavior appears congruent with the diver-

Table 3.—Interspecific comparison of male courtship behaviors.

Occurence	*gala*	*hend*	*junc*	*snod*	X^2	*P*
Total trials	21	12	23	10		
PM	21	12	22	10	1.90	1
FM	21	12	2	10	57.58	<0.0001
mP	19	9	0	0	49.38	<0.0001
sP	1	0	0	10	59.14	<0.0001
Po	0	0	21	0	57.58	<0.0001
Total courting time t_{tot} (s) (± SE)	101 ± 16	147 ± 36	50 ± 10	301 ± 72		

gence pattern in two male genital structures involved in the copulation process. In contrast, the variation in male courtship behavior contrasts with the ecological divergence into morphologically distinct high elevation and coastal dry species. Therefore, species with a similar habitat preference, which are highly similar in color pattern and in non-genital traits (De Busschere et al. 2012), share no similarities in male courtship behavior, and hence this observation does not suggest that these mating traits evolved as a byproduct of natural selection. Additionally, the variation in male courtship behavior is rather in disagreement with the phylogenetic relationships, as the more recently diverged *H. junco* and *H. snodgrassi* (~0.1 MY ago) are much more different in male courtship behavior than *H. heudrickxi* and *H. galapagoensis*, which diverged approximately 0.8 MY ago, albeit under low levels of gene flow (De Busschere et al. 2010). In sum, on Santa Cruz and San Cristóbal, parallel within-island speciation is only reflected in ecologically relevant traits and not in male courtship behavior. This incongruence indicates that, beside the similar and strong natural selection, different processes influenced the divergence of mating traits.

Weak prezygotic mating barriers.—Whether the above-mentioned interspecific differences have the potential to function as premating isolating mechanisms was investigated by performing interspecific mating trials. Beside the differences denoted in male courtship and morphology, we note that hitherto undescribed chemical and vibratory cues might also influence the outcome of these mating trials (Uetz & Roberts 2002; Roberts & Uetz 2004). Indeed, the latter might be expeeted, as palpal drumming dominates male courtship, and both drumming and push-up movements might result in vibrations being transmitted through the substrate. In general, female acceptance rate of courting males was low (10%), which was probably due to the use of wild-caught individuals. The latter refers to the possibility that wild-caught females were already fertilized in the field, leading to a possible rejection of courting males in the laboratory (Fernández-Montraveta & Ortega 1990; Rypstra *et al* 2003). Despite the generally small volume of data, which does not permit us to test for species-specific acceptance rates, females of *H. galapagoensis* accepted heterospecific males. Remarkably, despite clear differences in morphology, females of *H. galapagoensis* accepted heterospecific males of the genetically closely related sister species *H. hendrickxi*. Moreover, *H. galapagoensis* females also accepted heterospecific males from the distantly related but morphologically highly similar *H. junco,* which has a distinct male courtship (Poking). Therefore, despite clear interspecific differences in male courtship behavior and/or morphology, within and between-island acceptances were observed, which suggests that interspecific prezygotic mating barriers are weak for *H. galapagoensis*. This contrasts sharply with other wolf spider studies, in which distinct differences in courtship behavior serve as a strong prezygotic mating barrier (Den Hollander & Dijkstra 1974;

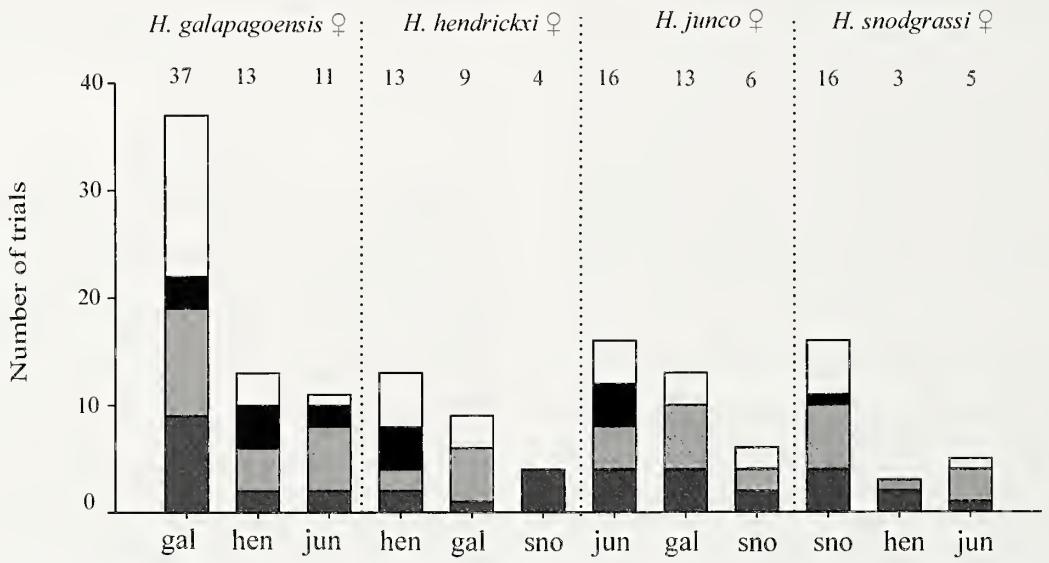

Figure 2.—Number of trials with no males courting (white), acceptance of courting male (black), aggression against courting male (light gray) and no response to courting male (dark gray). Total number of trials are noted above bars.

Uetz & Denterlein 1979; Töpfer-Hofmann et al. 2000; Chiarle et al. 2010). Furthermore, it is remarkable to note potentially weak prezygotic mating barriers, taking into account the deep divergence times [*H. galapagoensis-H. hendrickxi*: ~0.8MY and *H. galapagoensis-H. junco*: ~1.6MY ago (De Busschere et al., 2010)] and strong ecotypic divergence. The lack of premating barriers has also been found between allopatric lineages of warbler finches on Galápagos, despite differences in song and a long divergence time (1.5–2 MY) (Grant & Grant 2002). Weak prezygotic barriers might be explained by the lack of or weak selection against hybridization due to low levels of gene flow, and hence a predominant divergence in allopatry (Coyne & Orr 1989). Therefore, females of *H. galapagoensis* were not forced to recognize heterospecific males. The lack of frequent interactions has also been suggested for the absence of strong mating isolation between stream and lake sticklebacks (Raeymaekers et al. 2010). Indeed, range overlap between both ecotypes on Santa Cruz and San Cristóbal might have been limited to periods of environmental and climatological change (De Busschere et al. 2010).

Despite the current spatial isolation, the potential for weak prezygotic mating barriers points out that *Hogna* species boundaries, especially of *H. galapagoensis,* could be fragile in the case of future secondary contact. Moreover, the potential for weak prezygotic reproductive barriers for *H. galapagoensis* in combination with the detection of ancient hybridization events between *H. galapagoensis* and *H. hendrickxi* (De Busschere et al. 2010) and of inter-island dispersal of *H. galapagoensis* (Fig. 1), suggest a potential role of within and between-island gene flow in the *Hogna* radiation. Further exploration of the potential role of gene flow should not be neglected in understanding the *Hogna* radiation on Galápagos, as hybridization among diverging populations might enhance the spread of adaptive genetic variation and as such catalyze adaptive divergence (Seehausen 2004; Barrett & Schluter 2008; Schluter & Conte 2009) and facilitate recurrent phenotypic evolution. However, the possibility of other mating barriers such as assortative mating related to habitat preference (Rundle et al. 2000; Boughman 2001), natural selection against migrants and hybrids (Hendry 2004; Nosil & Crespi 2004) and the role of mechanical and postzygotic isolation mechanisms, definitely needs further assessment.

In sum, this study provided an initial view of the role of behavioral isolation among habitat-specialized wolf spiders on the Galápagos and emphasizes the need for further assessment of the degree of reproductive isolation and the potential role of within and between-island gene flow to understand the *Hogna* radiation on the Galápagos.

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Facilitation of ground-dwelling wolf spider predation on mirid bugs by horizontal webs built by *Tetragnatha* spiders in organic paddy fields

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Abstract. Trait-mediated effects of predators can impact prey population dynamics by affecting prey behavior. The mirid bug *Stenotus rubrovittatus* (Matsumura) (Hemiptera: Miridae), a major insect pest in Japanese rice production, usually remains in the upper layer of paddies to feed on rice ears. However, the mirids are frequently trapped by horizontal webs of *Tetragnatha* spp. spiders, which are highly abundant in organic rice paddies, and fall to the bottom layers of paddies where they are preyed upon by ground-dwelling predators. It is hypothesized that *Tetragnatha* spp. spiders facilitate bug predation by wolf spiders through trait-mediated effects, in which their horizontal webs force the bugs onto or near the ground and thereby into the hunting zones of wolf spiders. Molecular gut-content analysis of 619 wolf spiders coupled with field measurements revealed that the number of wolf spiders that tested positive for mirid bug predation increased significantly with the density of *Tetragnatha* spp. spiders in the paddies. We also observed a positive relationship between *Tetragnatha* spp. abundance and total cover by their webs in paddies. We identified the potential for an unexpected interaction between an herbivorous insect pest and ground-dwelling spiders that usually inhabit different microhabitats in paddy fields by focusing on trait-mediated effects of webs built by *Tetragnatha* spp. Because spider webs occupy a certain proportion of the available space in terrestrial ecosystems, consideration of trait-mediated effects on interactions between flying insects and other predators may lead to a better understanding of local food webs.

Keywords: Ecosystem function, molecular-gut content analysis, natural enemy, pest management, trait-mediated effect

Predators affeet prey populations through both density-mediated and trait-mediated effects, which can extend throughout the food web (Werner & Peacora 2003; Schmitz 2010). Trait-mediated effects are mainly mediated by behavioral changes in prey or other organisms, including reductions in feeding time (Griffin & Thaler 2006) and emigration from a particular microhabitat (Nakasuji et al. 1973; Losey & Denno 1998). These effects can cause indirect changes in the biomass, diversity (Schmitz 2010) or quality of food that is available to predators (Griffin & Thaler 2006) in both natural and agricultural ecosystems.

The webs woven by spiders might not only function as passive traps that catch prey but could also interfere with insect flight behaviors by forcing individuals to avoid spider webs (Rypstra 1982; Craig 1986; Blackledge & Wenzel 1998) or through attraction by web silk decorations (Craig & Bernard 1990; Tso 1998; Watanabe 1999). These trait-mediated effects of webs may significantly alter the biological interactions between flying insects and other predators, but few studies have explicitly tested this possibility.

Spiders are ubiquitous predators in rice paddies. In northern Japanese organic paddies that are managed using few or no chemical applications, *Tetragnatha* spp. (Araneae: Tetragnathidae), horizontal web weavers that live in the canopies of rice paddies, are conspicuously abundant (Oyama et al. 2005; Amano et al. 2011). The most serious threat to rice production in this area is grain discoloration caused by *Stenotus rubrovittatus* (Matsumura) (Hemiptera: Miridae). They prey on ears of wild poaeeous plants and cultivated rice. From source populations in meadows and fallows (Yoshioka et al. 2011), adult bugs spill over into rice paddies after rice plants start heading and infest rice grains (Takada et al. 2012). Our observational study (Takada et al. 2012) indicated that *Tetragnatha* spp. decrease the abundance of the mirid bug *S. rubrovittatus* and reduce the amount of damage caused by the mirid, suggesting that the spiders act as a natural enemy against the bugs.

In our study paddies, *S. rubrovittatus* is frequently observed falling to the bottom layer of the paddies after becoming tentatively trapped by horizontal webs of *Tetragnatha* spp. in the rice canopy. The webs seem to be too fragile to catch the bugs and are better adapted for catching small flies, such as chironomids (Kato et al. 2003), which are considerably smaller than the mirids. Bugs that fall to the bottom layer are exposed to ground-dwelling predators such as wolf spiders (Lycosidae), which hunt on the ground or in the bottom layer (e.g., Kiritani et al. 1970), and are likely to be preyed upon by them. The mirid bugs usually stay and feed on ears of rice in the uppermost vegetation layer (Takada et al. 2012).

This study was conducted to test the hypothesis that horizontal webs woven by *Tetragnatha* spp. enhance *S. rubrovittatus* predation by wolf spiders, which are abundant predators in the bottom layer. We analyzed relationships between mirid bug predation and measured densities of *Tetragnatha* spp. spiders, total coverage of horizontal webs, and the number of wolf spiders. Molecular gut-content analysis using DNA markers specific for *S. rubrovittatus* (Sheppard & Harwood 2005; King et al. 2008; Kobayashi et al. 2011) facilitated our evaluation of the magnitude of wolf spider predation.

METHODS

Study sites.—The study was conducted in Osaki City, Miyagi Prefecture, Northern Japan (38°37′N, 141°07′E) in August 2008. Annual precipitation in the area was 1126 mm,

and mean temperature in August 2008 was 22.5°C (Japan Meteorological Agency 2010). In this area, community-based, biodiversity-friendly farming activities have been expanding recently (Kurechi 2007).

Field survey.—Nineteen paddy fields that had similar management without the application of chemical herbicides or insecticides were chosen within an area of about 20 km^2. Two spider groups, *Tetragnatha* spp. and wolf spiders, were the most abundant spider groups in the paddy fields chosen for this study (Oyama and Kidokoro 2003; Takada et al. 2012). A field survey was conducted in August 2008, when rice ears were fully emerged and the mirids were at peak density. The densities of *Tetragnatha* spp. and *S. rubrovittatus* were measured at the center of each paddy field using net sampling; 20 sweeps were performed with a 36-cm-diameter sweeping net. At the same time, chironomid abundance was also measured because chironomids are known to be an important alternative prey for wolf spiders in paddy fields (Settle et al. 1996; Ishijima et al. 2006), and we hypothesized that abundant alternative prey interfere with mirid predation by wolf spiders (Harwood et al. 2004; Kuusk & Ekbom 2010; Öberg et al. 2011). To estimate the availability of alternative prey for wolf spiders, body lengths of chironomids were measured to the nearest 0.1mm with a measuring ocular on a stereo microscope, and their body mass was calculated in each field using the equation mass = 0.00305 × (body length)$^{2.62}$ (Rogers et al. 1976). Body mass was used instead of density because body length of these prey varied widely. Wolf spider density was estimated by direct counting. We walked through the rice hills (sheaves consisting of several rice stems) in a straight line along a row of 15 rice hills per field. When wolf spiders were observed in and around hills, the individuals were counted.

It was difficult to measure web coverage in all of the 19 study fields because *Tetragnatha* spp. build webs mainly from dusk till dawn (Kiritani et al. 1972; Tahir et al. 2009). Therefore, we used *Tetragnatha* spp. density, measured during the net sampling, as an index of the coverage of their webs in each paddy field, after testing for a positive relationship between the densities of spiders and webs in eight study fields (see Statistical Analyses). The coverage of *Tetragnatha* spp. webs was measured in three quadrats (1 × 1 m) set in the center of each of eight study paddy fields just before dusk or just after dawn. To enhance the clarity of webs for observation, the webs were misted with water using an atomizer. The mean area of webs in the three quadrats was used as an index of the coverage of *Tetragnatha* spp. webs in each paddy field.

Prey detection evaluation.—At the center of each study site, approximately 30 wolf spiders larger than 4 mm in body length were collected. We assumed that spiders smaller than this critical size could not consume the bugs, which had adult body sizes of 4.22 ± 0.49 (mean ± SD) mm (n = 32). Collected spiders were transferred to vials containing 80% ethanol, identified to species as well as sex and developmental stage (adult or juvenile), and placed in a freezer at −20°C until laboratory gut-content analysis. We investigated whether each spider had recently fed on *S. rubrovittatus* by testing for the presence of bug DNA in their gut contents (Kobayashi et al. 2011). In short, DNA was extracted from the abdomen of the spiders. A 250 bp DNA fragment from the mitochondrial cytochrome c oxidase subunit I (*COI*) gene of the target prey was amplified by polymerase chain reaction (PCR) using *S. rubrovittatus* specific primers. Amplified DNA was verified by electrophoresis in agarose gel. The proportion of individuals that tested positive was estimated by dividing the number of individuals that tested positive for bug DNA by the total number of spiders analyzed in each study field.

Data for abundances of spiders, *S. rubrovittatus*, and chironomids, and the proportion of individuals that tested positive for bug DNA in wolf spiders were obtained from a dataset created during our previous study (Kobayashi et al. 2011). However, the goals and hypotheses of the present study are different from those of Kobayashi et al. (2011).

Statistical analyses.—To test whether horizontal web cover increased with the abundance of *Tetragnatha* spp., a simple linear regression analysis was performed using eover as a dependent variable and *Tetragnatha* spp. density as an independent variable. We then applied a multiple logistic regression analysis to test whether increases in the abundance of *Tetragnatha* spp. were associated with increases in the proportion of wolf spiders testing positive for *S. rubrovittatus* DNA; we used the abundances of *Tetragnatha* spp. spiders, wolf spiders, and the bugs, and chironomid biomass as independent variables. To check for collinearity between the independent variables, tolerance values were compared to the critical value of 0.1 (Quinn & Keough 2002). All statistical analyses were performed using R for Windows 2.13.1 (*R* Development Core Team 2011).

RESULTS

The dominant *Tetragnatha* species were *Tetragnatha caudicula* Karsch 1879, *Tetragnatha extensa* Linnaeus 1785, *Tetragnatha maxillosa* Thorell 1895 and *Tetragnatha praedonia* L. Koch 1878. A positive relationship was found between the density of *Tetragnatha* spp. and the area covered by their horizontal webs, although it was marginal ($F_{1,6}$ = 4.814, P = 0.071; Fig. 1). The highest web cover in the study fields was 25.8%.

All of the wolf spiders colleeted during the study period were *Pirata subpiraticus* Bösenberg & Strand 1906. In total, 691 *P. subpiraticus* individuals from 19 study fields were analyzed for gut content. A multiple logistic regression analysis showed that the proportion of individuals that tested positive the bug DNA was related positively to the density of *Tetragnatha* spp. and negatively to chironomid biomass (Table 1, Fig. 2). All tolerance values were greater than the critieal value of 0.1, indicating that there was no significant collinearity between the independent variables (Table 1).

DISCUSSION

Molecular gut-content analysis revealed that mirid predation by wolf spiders increased with the density of *Tetragnatha* spp. in paddy fields. This partially supports our hypothesis that *Tetragnatha* spp. spiders facilitate bug predation by wolf spiders through trait-mediated effects. We collected wolf spiders for the gut-content analysis during the daytime, although feeding activity of these spiders seems to be more intensive from dusk till dawn (Kiritani et al. 1972), as does that of the bugs (Butler 1972; Mueller & Stern 1973).

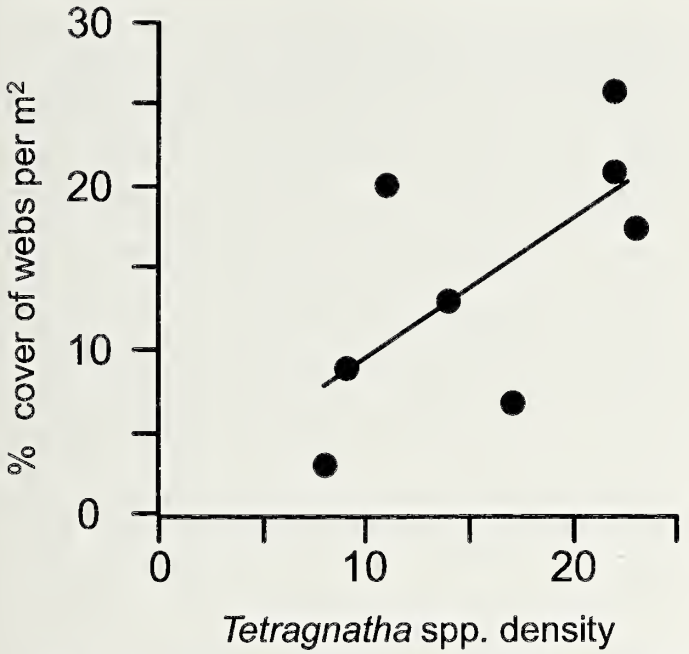

Figure 1.—Relationship between the density of *Tetragnatha* spp. spiders and the area covered by their webs per m^2. Estimated linear regression line ($y = 0.850x + 1.043$; $r^2 = 0.445$) is shown.

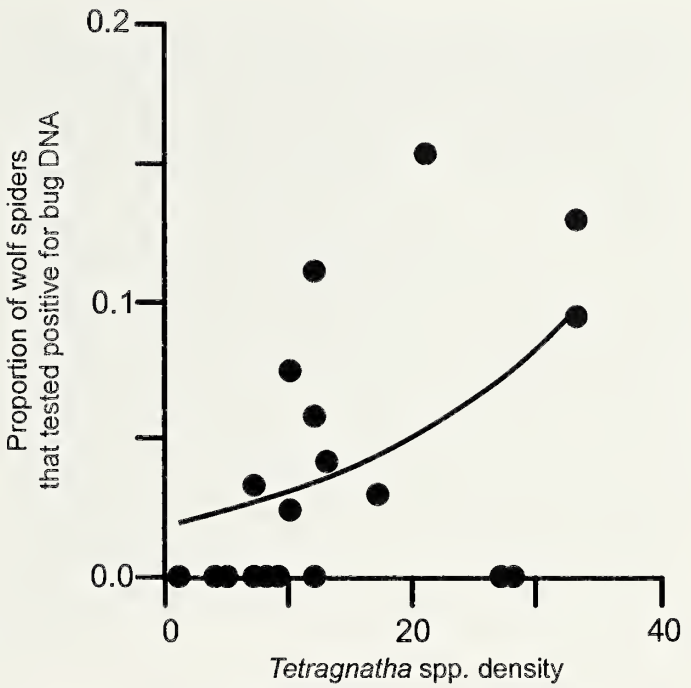

Figure 2.—Relationship between the density of *Tetragnatha* spp. spiders and the proportion of individuals tested positive for bug DNA in wolf spiders. Estimated logistic regression line is shown.

However, the sampling is unlikely to underestimate the spider predation on the bugs because the detection half-life (e. g., Chen et al. 2000) of *S. rubrovittatus* DNA in wolf spiders' guts was long (approximately 3.4 days at 25°C; Kobayashi et al. 2011). Our observation that their horizontal webs force the bugs to relocate onto or near the ground, into the hunting zone of wolf spiders (M.B. Takada pers. observ.) would be the primary cause of this trait-mediated effect. The positive relationship between *Tetragnatha* spp. density and the coverage of their webs strengthens this inference, although it would be more accurate to take *Tetragnatha* spp. web coverage measures of all of the 19 study fields and test the relationship between mirid bug predation by wolf spiders and the web coverage directly. In addition, in a future study, we will test whether the horizontal webs of *Tetragnatha* spp. cause bugs to drop to the surface of the water, and whether their effects influence bug density and bug-induced crop damage in paddy fields.

Chironomid biomass decreased mirid predation by wolf spiders. It is known that dipterans such as chironomids are important alternative prey for spiders in paddies (Ishijima et al. 2006; Tahir & Butt 2009). Abundant alternative prey may interfere with mirid predation by wolf spiders. Previous studies have also revealed negative relationships between insect pest consumption by spiders and availability of alternative prey in crop fields (Harwood et al. 2004; Kuusk & Ekbom 2010; Öberg et al. 2011).

The top-down effect of *Tetragnatha* spp. spiders on the bugs and the amount of crop damage that was demonstrated in our observational study (Takada et al. 2012) can be attributed to the trait-mediated effect of the horizontal webs of *Tetragnatha* spp. In the bottom layer in paddy fields, there are many generalist predators besides wolf spiders that are larger than the bugs, including other hunting spiders, such as *Pachygnatha clercki* Sundevall 1823 (Oyama et al. 2005; Takada et al. 2012), and water striders, such as *Gerris* spp. (M.B. Takada, pers. observ.). These predators might also prey upon bugs when they fall to the ground after being trapped by *Tetragnatha* spp. webs. Predation by ground-dwelling predators on aphids that fall from plants has been reported in alfalfa fields (Losey & Denno 1998).

The enhancement of wolf spider predation on the mirid bugs by *Tetragnatha* spp. spider webs can be classified as a synergistic or substitutable effect (Schmitz 2007) between two generalist predators on the bugs. Although previous studies stressed that enhancement of biological control functions by increasing diversity of generalist predators is unlikely or

Table 1.—Multiple logistic regression results and tolerance values between independent variables.

Variables	Tolerance values	Estimate	SE	χ^2	P
Tetragnatha spp. density	0.466	0.079	0.025	9.843	0.002
Wolf spider density	0.936	0.012	0.018	0.478	0.489
Chironomid biomass	0.614	−0.021	0.010	8.897	0.003
Bug density	0.738	0.016	0.009	3.757	0.053

limited due to antagonistic interactions, such as intra-guild predation (e.g., Finke & Denno 2004; Denno et al. 2004), we have demonstrated a circumstance in which two spider groups do not spatially share habitat domains (Schmitz 2010). In our system, *Tetragnatha* spp. inhabit webs in the uppermost layer, whereas wolf spiders hunt on the ground or in the bottom paddy layer.

We found the potential for an unexpected interaction between an herbivorous insect pest and ground-dwelling spiders that usually inhabit different microhabitats in paddy fields by focusing on trait-mediated effects of webs built by *Tetragnatha* spp. Since *Tetragnatha* spp. spiders are also dominant in riparian ecosystems (Henschel et al. 2001; Kato et al. 2003; Iwata 2007) and usually build horizontal webs at the water surface, the trait-mediated effects of their webs might affect relationships between terrestrial and aquatic food webs (Nakano & Murakami 2001) by subsidizing terrestrial prey to aquatic predators. As spiders are ubiquitous predators in terrestrial ecosystems (Wise 1993), their webs should occupy a certain proportion of the available space. Therefore, consideration of the trait-mediated effects of spider webs on interactions between flying insects and other predators may lead to a better understanding of local food webs.

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Specificity of attraction to floral chemistry in *Misumenoides formosipes* crab spiders

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Abstract. Although our understanding of arachnid olfactory physiology remains relatively limited, studies continue to reveal the importance of chemical cues for many spider behaviors. Olfactory cues for detecting prey, navigating to foraging sites, or finding mates might be especially beneficial to cursorial and ambush spiders living in structurally complex habitats. Previous field results suggested that volatile plant chemical cues were important in *Misumenoides formosipes* Walckenaer 1837 (Thomisidae) navigation and led us to design olfactometer bioassays to test this hypothesis in the laboratory. In our olfactometer trials, crab spider males were attracted specifically to the floral scent of *Rudbeckia hirta* (a species on which *M. formosipes* is commonly found in the field), but not to volatiles from foliage of the same plant species nor to volatiles from foliage of *Morus rubra*. Male spiders also failed to display any attraction to the floral scent of *Daucus carota*, even though they commonly reside on that plant in the field. Female *M. formosipes* did not move toward *R. hirta* inflorescences as a first choice over a control, although they did spend more time in the olfactometer arm with the *R. hirta* treatment. Males' use of olfactory cues to locate *R. hirta* inflorescences should increase encounters with potential mates, given that females in our population are found on that substrate more predictably than on any other.

Keywords: Floral scents, navigation, olfactometer, plant volatiles, spider olfaction, Thomisidae

Studies of the olfactory capacities of arachnids have lagged behind those of other arthropods, especially insects. The early recognition of antennae as a primary location of chemoreceptors in mandibulates and the absence of any clearly homologous structures in arachnids might in part account for this discrepancy. At this time, relatively few reports exist on the receptor anatomy and physiology of olfaction in spiders (e.g., Dumpert 1978; Foelix 1985, 2011). However, valid claims for the olfactory capacities of these animals come from demonstrations of behavior consistent with the reception of volatile chemicals, with studies combining behavior and receptor physiology being especially instructive (Tichy et al. 2001; Jiao et al. 2011).

Across spider families, there is substantial behavioral evidence for the existence of sex pheromones—either contact or air-borne or both (e.g., Schulz 2004; Gaskett 2007; Rypstra et al. 2009). Kairomones have been implicated in spiders' abilities to locate and discriminate among prey species, as well as avoid predators (Allan et al. 1996; Kaspi 2000; Hostettler & Nentwig 2006; Schonewolf et al. 2006; Cross & Jackson 2010). Olfactory or gustatory cues also enable spiders to find nectar sources (Patt & Pfannenstiel 2008), optimal hunting sites (Heiling et al. 2004; Junker et al. 2011) and substrates with greater prospects for locating mates (Stellwag & Dodson 2010). Among amblypygids, Hebets & Chapman (2000) recorded electrophysiological responses to a tremendous variety of volatile chemicals in the antenniform legs of one tropical species, and olfactory cues alone were sufficient for kin discrimination in a social species (Walsh & Rayor 2008).

Spiders that capture prey by stealth as opposed to webs might be especially likely to use chemical cues (animal kairomones and plant secondary compounds) to aid in locating prey, hunting sites or mates. For example, exposure to plant volatiles increased the number of *Thomisus spectabilis* Doleschall 1859 (Thomisidae) individuals attracted to inflorescences compared with visual cues alone (Heiling et al. 2004). Other *Thomisus* species were attracted to traps baited with eugenol, a component of many floral bouquets (Krell & Kramer 1998). Finally, males of the crab spider *Misumenoides formosipes* Walckenaer 1837 moved toward black-eyed susan (*Rudbeckia hirta* L.) inflorescences, the substrate upon which females were most commonly found, at a higher frequency when floral volatiles were available as opposed to visual cues alone (Stellwag & Dodson 2010).

The latter result led us to the present study in which we tested whether or not *M. formosipes* would navigate toward the chemical signatures of plants in the absence of associated visual and tactile cues. Laboratory bioassays were conducted in Y-tube olfactometers to address the following questions: 1) Are male *M. formosipes* attracted to plant volatiles from either the inflorescences or the foliage of *R. hirta*? 2) Are males attracted to volatiles from the inflorescences of Queen Anne's lace (*Daucus carota* L.)? 3) Are males attracted to volatiles from an arbitrarily chosen plant within their habitat [foliage of mulberry (*Morus rubra* L.)]? 4) Are female *M. formosipes* attracted to volatiles from the inflorescences of *R. hirta*? One of us (GND) has studied this population for many years and routinely found *M. formosipes* males and females hunting from the inflorescences of *R. hirta* and *D. carota* more than from any other substrates. If adult males use plant scents to locate females, we predicted that volatiles from these species should be attractive.

METHODS

Study organism.—*Misumenoides formosipes* is an ambush predator that feeds primarily on insect visitors to inflorescences (Beck & Connor 1992; Dodson & Beck 1993), but males also ingest nectar as a secondary energy/water source (Pollard et al. 1995). Our study population is on a managed preserve in Delaware County, Indiana, containing habitats recently converted to prairie as well as successional fields and a forest patch. The spiders occur on a wide variety of flowering plants at the forest edges and in wildflower fields, with black-eyed susans (*Rudbeckia hirta*), brown-eyed susans (*R. triloba* L.), chickory (*Cichorium intybus* L.), and Queen Anne's lace (*Daucus carota*) the most predictable species on

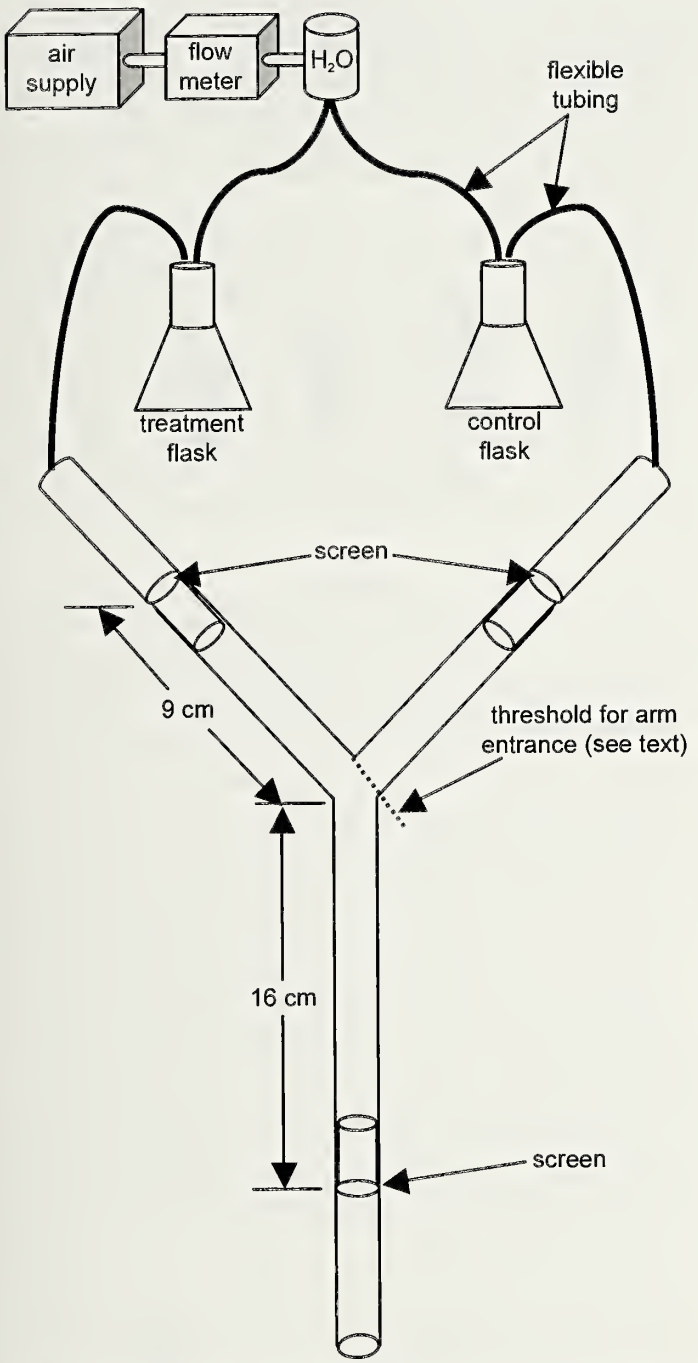

Figure 1.—Design of olfactometer used for biassays. Linear dimensions labeled indicate the distance from the mesh retaining screen behind the spider starting point to the point where each arm diverges and then the distance from the start of an arm to the screen preventing passage out of the olfactometer. The dotted line indicates the threshold a spider had to cross to be counted as having moved into the left or right arm.

which the late-instar juveniles and adults can be located (G.N. Dodson pers. obs.).

Males molt to the adult stage before females and begin searching the habitat for penultimate instar females that are nearing their own molt (Dodson & Beck 1993). The population sex ratio is strongly male-biased as the adult period begins [as high as 63% males in early August samples (Dodson & Stellwag unpubl. data)]. Adult males live only 2–3 weeks, after which females continue to hunt until laying eggs that hatch in the fall and give rise to overwintering spiderlings.

Olfactometer set-up and protocol for all trials.—Three olfactometers were assembled for each set of trials and laid out in parallel over white paper. Each olfactometer (Fig. 1) consisted of a glass Y-tube (Analytical Research Systems, Inc., Gainesville, FL), with both of its arms connected to a 50 ml treatment or control flask via flexible tubing. Air from a single source flowing at 20 ml/min was bubbled through 300 ml distilled water and this humidified air then traveled through both sides of the olfactometer before exiting through the base of the Y-tube. Factory inserted screens (Fig. 1) prevented spiders from moving out of the olfactometer.

For each bioassay trial, we placed one of the four treatments (*R. hirta* inflorescence, *R. hirta* leaves, *D. carota* inflorescence, *Morus rubra* leaves) into one of the two flasks along with 2 ml of water. The control flask contained only the 2 ml of water. We cut a single, typical inflorescence (ca. 5 – 6 cm diameter) for each trial in the *R. hirta* bioassays and took care to use a similar amount of plant material, whether inflorescence or leaves, across all treatments. The stem of the inflorescence or the petioles of the leaves were inserted into the water, with the remainder of the plant material resting above the water. We alternated the treatment flask between the left and right sides of the olfactometer in a pattern that resulted in equivalent numbers of trials conducted with plant material on each side. We positioned and shielded the flasks to eliminate the possibility of any visual cues for the spiders.

Spiders for the bioassays were collected daily from the field, held in vials with moist filter paper, and used in trials within 24 h or rarely 36 h. At the start of each trial, we allowed a spider to move from the vial to the introduction tube of the olfactometer on its own and gently prodded it only if it did not transfer after several minutes. Each spider was used in a single trial and then returned to the field site the next day. We collected new spiders well away from release sites, so there was a very small chance that we collected any male more than once. Trials were conducted between 25 July and 12 August in 2008, 2009, 2010, and 2011.

The temperature of the trial room varied minimally around 23° C. Standard florescent light bulbs remained on during all trials. All olfactometer glassware was washed using a bottle brush and detergent, rinsed thoroughly, and oven-dried between trials, with particular attention paid to clearing all residual silk from spider movements.

We started a set of trials each day at ca. 08:00 and a second set at ca. 20:00 and ran both undisturbed for 10 h. This trial duration was both expedient (typically no more than the required six males could be located within the search time available each day) and conservative (we had no way to predict beforehand how much time might pass before spiders began to move). We completed 30 trials for each treatment (two sets of 30 trials were conducted for males with *D. carota* inflorescences – see below). All trials were recorded with a digital video camera and then played back to determine 1) the time from the start of the trial until the spider entered an arm for the first time (= latency), 2) which arm (treatment or control) was visited first and 3) the total time spent within each arm during the trial. To be recorded as having entered an

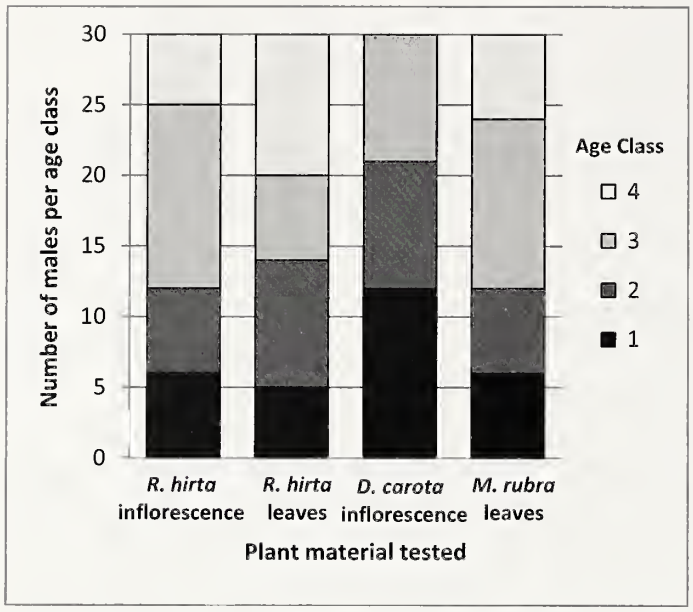

Figure 2.—Number of males used from each age group category across the four sets of trial treatments. Age classes represent the dates that males were collected and used in the trials from relatively youngest (Group 1) to oldest (Group 4). Collection dates were 30 July–2 August (Group 1), 3–5 August (Group 2), 6–8 August (Group 3), and 9–12 August (Group 4).

arm of the olfactometer, the entire body of the spider had to cross the threshold of the arm (see Fig. 1).

Protocol specific to male trials.—Adult males were collected from *R. hirta*, *R. triloba*, and *D. carota* plants, primarily from inflorescences, but from other parts of these plants as well. We did not record the exact proportion collected from each of the plant species, but a majority came from *Rudbeckia*. Following completion of a full set of trials with each of the four treatments, we conducted a second set of 30 trials with *D. carota* inflorescences using males collected exclusively from *D. carota*. The purpose was to assess whether or not males known to have had experience with *D. carota* as a substrate might behave differently when exposed to volatiles of that plant in our olfactometer. Thus, we addressed the possibility that males in the initial trials showed no attraction to *D. carota* (see Results), because at least some of them might not have experienced that plant in the field.

In anticipation of a potential effect of adult male age on the vitality of individuals, and thus their tendency to move within the olfactometer, we spread the four treatments across the trial dates as evenly as possible year to year. Although we could not know the age of a given male in the field, we assumed that the average adult age (time since adult molt) increased daily once molting began, since most of the initial adults in the population would still be alive as newly molted males entered the cohort. The only exception to this trend might be within the first few days if freshly molted individuals outnumber the adults from previous days. For the purpose of analysis, we divided the trial males into four groups based on date of collection (Fig. 2) and used these as a proxy for "relative age" of adult males.

Protocol specific to female trials.—Females were collected from *R. hirta*, *R. triloba*, and *D. carota* plants, exclusively from inflorescenses. All of the females were at least penultimate instar, and some of the last ones collected may have been adults (genital morphology was not examined in order to avoid extensive handling). We collected only females exhibiting the behaviors of active foraging to avoid the use of individuals in a molting phase, which would be less likely to move in the olfactometer.

Statistical analyses.—The data recorded for the time spent by spiders in the treatment and control arms, time spent in first choice and second choice arms, as well as male latency times were not normally distributed. Therefore, a Box Cox transformation was performed followed by the use of parametric tests wherever normality was achieved and nonparametric tests otherwise. We tested our data for normality with the Wilk-Shapiro test and homogeneity of variances with Levene's test. All statistical analyses were performed using IBM SPSS Statistics v. 19. All *P*-values are two-tailed with an alpha level of 0.05.

RESULTS

Effects of experimental design.—We first examined the combined trial outcomes for the initial four treatments with males ($n = 120$) to determine if the position of the treatment, starting time of the trials, or relative age of the spiders had unintended impacts as factors in the experimental design. None did as revealed by statistically equivalent frequencies for first choice of the treatment arm whether it was on the left or right of the olfactometer (Pearson $\chi^2 = 1.17$, $df = 1$, $P = 0.28$), morning or evening trial starts (Pearson $\chi^2 = 0.57$, $df = 1$, $P = 0.45$) or date of collection of spiders used in the trials (Pearson $\chi^2 = 3.2$, $df = 3$, $P = 0.36$). Therefore, these parameters were not included as variables in the final analyses.

We also considered whether or not males collected from the field during our final days of testing (and therefore older on average) might be less "active" and potentially provide different results for that reason alone. Using latency (i.e., time from the beginning of a trial until an initial choice of olfactometer arm) as an indicator of activeness, we failed to detect a relationship between relative age and latency (Pearson correlation coefficient = 0.14, $df = 3$, $P = 0.12$) using the complete data set. However, the removal of a single outlier for latency (30% larger than any other value) produced a significant correlation upon reanalysis (Fig. 3; Pearson correlation coefficient = 0.184, $df = 3$, $P = 0.046$). As stated above, we intentionally distributed the four treatments across the trial dates as a control for this anticipated effect. Thus, we feel that any age related effects would have had little if any influence on our overall findings. We offer further discussion of this issue below.

Male responses to volatiles.—*Misumenoides formosipes* males entered the *Rudbeckia hirta* inflorescence treatment arm prior to the control arm of the olfactometer significantly more often than by chance (Fig. 4, binomial exact probability = 0.0014). They also spent more time in the *R. hirta* inflorescence treatment arm, although not at a statistically significant level (Fig. 5, Mann Whitney test, $z = 1.8$, $P = 0.069$). There were no significant differences in the frequencies with which *M. formosipes* males chose the treatment versus the control arm first for *R. hirta* foliar treatment (binomial exact probability = 0.36), *D. carota* floral treatment (binomial exact

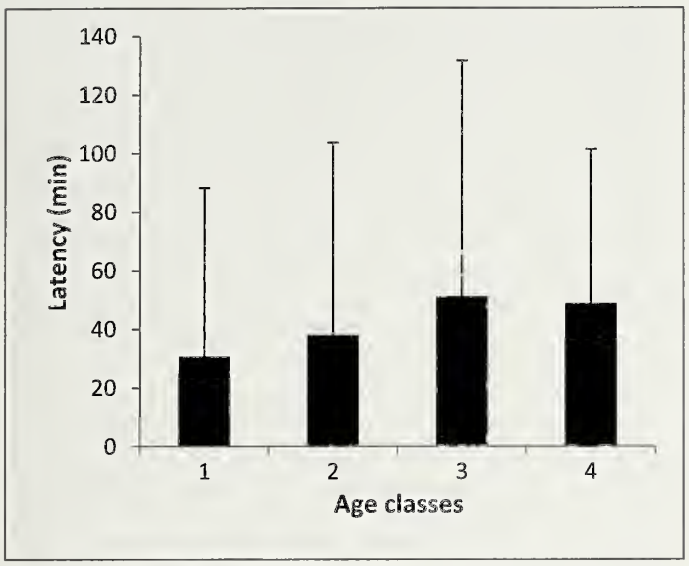

Figure 3.—Mean latency values for each male age class for the original four sets of trial treatments combined (n = 120, error bars display one standard deviation; one outlier was removed from group 1). Latency was defined as the time from the start of a trial until the moment a male crossed into either of the two olfactometer arms. See Methods for a description of the rationale for this approximation of relative ages.

probability = 1.0) and *M. rubra* foliar treatment (binomial exact probability = 0.098) (Fig. 4). Likewise, the proportional time spent in treatment and control arms did not differ for these three treatments (Fig. 5, Mann Whitney tests: *R. hirta* foliage, z = 0.57, P = 0.56; *D. carota* floral, z = 0.64, P = 0.52; *M. rubra* foliage, z = 0.87, P = 0.38).

Test for effect of prior experience with floral volatiles.—Males collected exclusively from *D. carota* inflorescences exhibited the same non-preference for *D. carota* floral treatment as did the males in the original trials with that plant species. Seventeen of 30 males moved into the inflorescence arm before the control arm (binomial exact probability = 0.54). Thus, we found no evidence to suggest that familiarity with a particular substrate influences subsequent responses to its

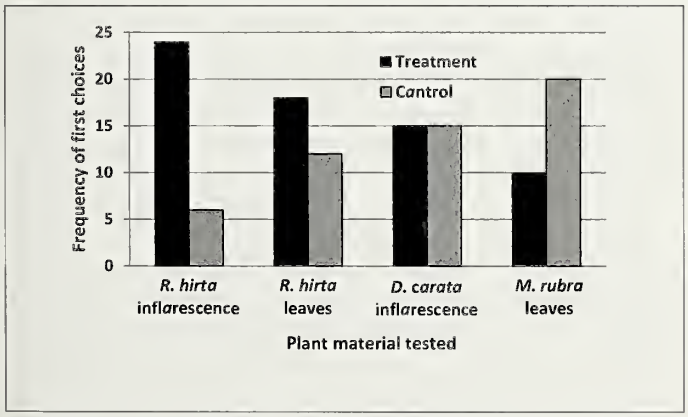

Figure 4.—Frequencies of males choosing to move first into the treatment versus the control arm of the olfactometer. Only the *R. hirta* inflorescence treatment arm was chosen first significantly more often than the water control. N = 30 for each treatment.

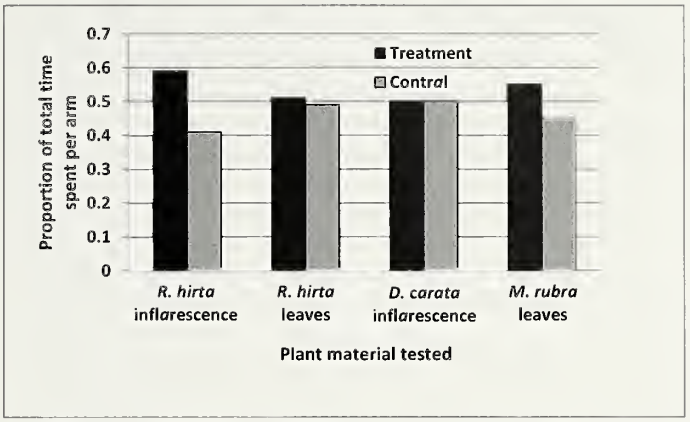

Figure 5.—Time spent by males in the treatment arm versus the control arm as proportions of the total time spent in both. No significant differences were found between treatment and control arm times based on 30 trials for each of the four treatment types. Proportional values shown correspond to the following average total time per trial in the arms: *R. hirta* inflorescence = 171.8 min, *R. hirta* leaves = 395.1 min, *D. carota* inflorescence = 368.0 min, *M. rubra* leaves = 317.7 min.

chemical signature, further support for the lack of a behavioral response to any volatiles from *D. carota* inflorescences.

Female responses to floral volatiles.—Females showed no significant preference for the *R. hirta* floral treatment arm versus the control arm as a first choice (50% of 30 females entered the treatment arm first, binomial exact probability = 1.0). However, they spent significantly more time (61% of the total time in the two arms) in the arm with the floral treatment [medians and first quartiles: 192.1 (108.9) min for treatment arm, 72.9 (26.0) min for control arm; Mann Whitney test, z = 2.07, P = 0.036].

DISCUSSION

Studies are increasingly revealing the ways in which spider behavior is influenced by olfaction, particularly in foraging and mating systems (e.g., Hostettler & Nentwig 2006; Gaskett 2007; Cross & Jackson 2010). We submit that non-web building spiders should benefit the most from the use of airborne chemical cues for navigation to hunting sites and potential mates. Locomotion represents an obviously large part of their energy expenditure, and visual targets might be difficult to locate within the complex three-dimensional space occupied by most of these species. Variety in the use of chemical cues for hunting is illustrated by a cursorial spider that finds its ant prey by detecting their alarm pheromone (Allan et al. 1996) and a nectarivorous ghost spider that locates its nectar source via scent cues (Patt & Pfannenstiel 2008). In field trials on *M. formosipes*, the availability of chemical cues in addition to visual ones increased the attraction of males to the kind of inflorescences that often harbor females (Stellwag & Dodson 2010), which led us to the hypotheses tested herein.

Male responses.—The strong attraction of *M. formosipes* males to *R. hirta* floral volatiles in our laboratory trials was expected following the aforementioned field trial results (Stellwag & Dodson 2010). Likewise, it seems appropriate that 60% of the trial males moved toward the foliage of this

same plant, since that also would ultimately bring them toward *R. hirta* flowers. Finally, moving away from the *Morus rubra* plant volatiles is also unsurprising, given that these crab spiders depend on flower-visiting prey and *M. rubra* flowering occurs more than two months prior to these spiders becoming adults. Any direction that takes them away from a "wrong" choice might be efficient. The one result that contrasted with our expectations was the males' indifference to the odor of *Daucus carota*. Females are routinely found on this plant as juveniles and adults, so it would seem to be an appropriate target for mate-seeking males to pursue. Given that adult males mostly forego prey capture to hunt for mates, an additional incentive is that *M. formosipes* uses *D. carota* for nectar feeding (Pollard et al. 1995). Indeed, when returning them to the field after the indoor trials we often observed males spend several minutes in a nectar feeding posture on this plant species.

The lack of attraction to *D. carota* by the spiders in our initial trails made us consider whether past experiences of the males might have influenced their responses. Collecting males as they were encountered in the field meant that we took a minority from *D. carota*, making it possible they had no experience with that plant substrate. Perhaps they had not learned to "recognize" its chemical signature. At least one case of olfactory imprinting has been demonstrated in spiders. Punzo (2002) fed separate groups of a lynx spider an exclusive category of prey and found that they subsequently displayed a preference for odors matching their prey type. However, our follow-up olfactometer trials using males collected exclusively from *D. carota* revealed the same lack of attraction to that plant species as in the initial trials. At face value, these results indicate that the spiders may not locate all favored plant species by floral scents. We acknowledge, however, that the act of cutting the floral stems for our bioassays may have altered the production or release of chemical compounds compared with the intact plant.

Our finding that a coarse measurement of relative male age (i.e., timing of collection from the field) was a predictor of latency times was not surprising. Given their long distance travel in search of females coupled with male-male aggressive interactions over mating opportunities (Dodson & Beck 1993; Dodson & Schwaab 2001), we might expect male vigor to decrease with time. Notably, in the closely related *Misumena vatia* Clerck 1757, older males lost 70% of staged contests with younger males (Hu & Morse 2004). Any influence that this variable might have had on male behavior in our trials, however, should have been mitigated by our equitable distribution of trial types across the dates of spider collection. Indeed, the pattern for first choice of treatment versus control in the olfactometers did not vary among the relative age categories.

Female responses.—Crab spider species that forage by ambushing pollinators are logical candidates for exploiting floral scents to locate hunting sites. This is particularly expected of females since their fecundity ultimately depends on foraging success (Schmalhofer 2001; Morse 2007). Indeed, the choice of hunting substrates by female *Thomisus spectabilis* Doleschall 1859 depended on whether or not floral scents were made available to them (Heiling et al. 2004). When floral scents were presented, *T. spectabilis* chose the same inflorescences favored by one of their primary prey species, *Apis mellifera*. By contrast, our results were somewhat ambiguous regarding *M. formosipes* females' preferences for the floral scents of a plant on which they are commonly found. Females displayed no tendency to move first toward the *R. hirta* inflorescence over a water control, but they did spend significantly more time in the floral treatment arm during the trials. When Junker et al. (2011) gave *Misumena vatia* females the choice between inflorescences and leaves of five species in laboratory trials (via intact plant material as well as hexane extracts of the plant parts), they reported no significant preferences for floral over foliar options. The latter finding does not preclude floral scent attraction, however, since the lack of a preference between the two parts of the plant does not rule out equivalent levels of attraction to both. Trials with a control that contained no volatile plant chemicals would be necessary to rule out this alternative. At this point, it is not possible to draw generalizations on the olfactory tendencies of crab spider females at the subfamily level given the differing results reported in these three studies.

Why would *M. formosipes* females fail to exhibit the strong attraction displayed by males toward *R. hirta* floral scents? It was not due to differences in how quickly a decision was made in the Y-tube apparatus, as the latency times were virtually identical (67.8 ± 95.1 min for males and 65.4 ± 96.9 min for females, mean ± SD). Sexual differences in the species' life history may be a factor. *Misumenoides formosipes* is protandrous, with the peak in adult male molts occurring at least 1 wk prior to the earliest maturation of females (G. N. Dodson pers. obs.). Coincident with adulthood, male activity is focused on searching for potential mates, primarily penultimate females close to their adult molt (Dodson & Beck 1993), whereas females continue to operate as ambush predators and exhibit substantial site fidelity (Beck & Connor 1992). We can see how males would benefit from continuously seeking the next inflorescence until a female is located, which may require many meters of travel. Females, on the other hand, move only when a new foraging site is needed and are likely to find an appropriate inflorescence nearby – making visual cues potentially sufficient for guidance, at least during the day. Female olfactory tendencies at night need further investigation, however. Of the 15 trials during which females moved into the *R. hirta* inflorescence arm first, 10 were night trials (although lights stay on). V.R. Schmalhofer (pers. comm.) has determined that *M. formosipes* females often make their hunting site moves nocturnally.

Conclusions.—Are we prepared to argue that the chemical signature of a single plant species is the major navigational cue for *M. formosipes* males seeking mates? Our current answer has to be "no" given the many potential cues in this process that remain uninvestigated. However, we now have laboratory results corroborating the original field study findings (Stellwag & Dodson 2010) on the significance of this specific olfactory signal. As part of ongoing bioassay work, we removed any potential effect of the physical plant body and found that 70.5% of 17 males chose the whole chemical extract from the *R. hirta* inflorescence over a water control. We intend to isolate and characterize the compounds in the extracts that elicit responses from spiders.

Certainly, females are found on other plant species including *D. carota*, and males converge quickly around near-adult females on these substrates (G.N. Dodson pers. obs.). Further

olfactometer trials with additional plant species are warranted, including a protocol that uses intact flowers on whole plants. For now, however, we are left to assume that males in our population benefit by seeking *R. hirta* inflorescences because of greater prospects for finding potential mates there. *D. carota* inflorescences have always been abundant over the years at our field site, but a lower percentage of them harbor females compared to *R. hirta.* Females also remained only half as long on *D. carota* (5.8 ± 6.1 d) as on *R. hirta* (12.6 ± 8.8 d) during field surveys (A.G. Anderson & G.N. Dodson unpublished data). In a roll of the dice that would seem to make the latter a better search target.

Our focus on phytochemical cues for male searches should not be seen as an argument that pheromone release by *M. formosipes* females is not important in mate finding. Given the growing documentation of sexual pheromones in spiders (Gaskett 2007), it is a reasonable conjecture that *M. formosipes* females might advertise their locations to males. At this point, however, several observations suggest the lack of a pheromone. In trials in which marked males (n = 68) were placed in the proximity of penultimate-instar females who were within days of their adult molt (and thus the target of searching males), fewer than 3% of these males moved to the nearby females (G. N. Dodson unpublished data). When males were placed directly onto the inflorescence housing a penultimate female for male-male contest trials (Dodson & Schwaab 2001), their behavior indicated a failure to recognize her presence until they happened to make physical contact with her body. Lastly, D.H. Morse and coworkers have found no evidence of a sex pheromone in the related species *Misumena vatia* (Holdsworth & Morse 2000; Legrand & Morse 2000; Leonard & Morse 2006). Even if a female sex pheromone were eventually identified in this species, it would not alter our interpretation that phytochemicals are important cues given that males are attracted to flowers with no females present.

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Reflections on the tapetum lucidum and eyeshine in lycosoid spiders

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Abstract. In the lycosoid spiders, the secondary eyes possess a grate-shaped tapetum lucidum that reflects light, causing eyeshine when these spiders are viewed with approximately coaxial illumination. This guanine-based reflective surface is thought to increase visual capabilities in low light. We explored the eyeshine of the posterior medial eye in eight taxa of pisaurid and lycosid spiders. The taxa included four pisaurids: *Dolomedes tenebrosus* Hentz 1844, *D. triton* (Walckenaer 1837), *D. scriptus* Hentz 1845 and *D. vittatus* Walckenaer 1837; and four lycosids: *Gladicosa pulchra* (Keyserling 1877), *Hogna* sp. (cf. *Lycosa lenta* (Hentz 1844) sensu Wallace 1942), *Rabidosa punctulata* (Hentz 1844) and *Varacosa avara* (Keyserling 1877). We found that there were significant family- and species-level differences in both the reflected spectra and the intensity of reflection. Although the peaks of the reflected spectra were in the green range for all spiders, the mean peak was further toward the blue end of the spectrum for the lycosids than for the pisaurids. Variation among species (about 54% of the total variation) was dominated by *G. pulchra* (Lycosidae) and *D. vittatus* (Pisauridae), both of whose spectra peaked near yellow, vs. *V. avara* (Lycosidae), whose spectra peaked to the blue side of green. The lycosid spiders showed overall brighter eyeshine. However, when corrected for their larger eyes, the lycosid spiders' reflections were dimmer for their eye size than were those of the pisaurid spiders. These results demonstrate that the reflective qualities of the tapeta, and perhaps the absorptive qualities of other tissues and media that the light must traverse, vary widely among lycosoid spiders. This variation may signal both functional differences in visual capabilities and interesting developmental or selective histories within this clade.

Keywords: Lycosidae, Pisauridae, posterior medial eye, vision

Eyes provide information to animals based on the reflection, refraction and emission of light in their environment (Ghering 2004). It is clear that varied evolutionary histories of eyed taxa have shaped the great diversity of eye morphologies (Goldsmith 1990; Land & Fernald 1992). For example, among invertebrates, spiders are unusual in that they possess eyes that function by corneal refraction (Land 1985; Land & Fernald 1992). Spiders are also unusual because they typically have eight eyes, where the primary eyes and the secondary eyes are specialized for different functions: the anterior medial pair of eyes (the primary eyes) are adapted for image formation (Land 1985), and the secondary pairs of eyes (anterior lateral, posterior medial and posterior lateral) are adapted for motion detection (Blest 1985; Land 1985; Land & Nilsson 2001; Neuhofer et al. 2009).

Across spider taxa, the relative importance and optimization of different aspects of vision are quite varied. Although orb-weavers are thought to have weak visual acuity (Land & Nilsson 2001), hunting spiders are reported to have image formation comparable (relative to body size) to human eyes (Land 1985). Even in the hunting spiders, however, eye use varies. For example, the ogre-faced spiders have posterior medial (PM) lenses that optimize light sensitivity (Blest & Land 1977), whereas in other hunting spiders image formation is optimized. In the salticids, the anterior medial pair of eyes is the largest and has the greatest resolution and sensitivity (Land 1969). However, the maximal resolution in lycosoids is in their largest eye pair, the posterior median eyes (PMEs; Homann 1931, as cited in Yamashita 1985; Williams 1979). The relative size and sensitivity of the eye pairs suggest that the PMEs are particularly important to the lycosoid spiders (Pirhofer-Walzl et al. 2007).

Probably because of the lycosoid spiders' crepuscular or nocturnal habits (Ortega-Escobar 2002), they possess several adaptations that enhance the function of the secondary eyes in low light conditions. The wide aperture of their lenses increases sensitivity in low light (Land & Nilsson 2001) and, as in many other families, these spiders adjust neural sensitivity on a diel basis to make use of available light by maximizing neural sensitivity in dark hours (Blest & Day 1978; Yamashita 1985). Also like many other spiders, lycosoids possess a tapetum lucidum ("silvery carpet"), a surface that reflects light back through the retina thereby increasing the likelihood that any photon will be captured by a photoreceptor (Schwab et al. 2002).

Spider tapeta fall roughly into three categories based on their morphology, but all of the lycosoids share the property of having a grate-shaped tapetum (Homann 1931, as cited in Land 1985; Land 1985). In this kind of tapetum, multilayers of guanine form strips of reflectors that underlie the rows of receptors (Land 1985; Oxford 1998), thus reflecting a "grate" pattern. These arrays often result in overlap of neural receptors, an overlap that reduces the resolving power of the eyes but maximizes their sensitivity to light (Blest & Day 1978). Grate-shaped tapeta can also facilitate navigation by detection of the polarization of light (Dacke et al. 2001; Warrant & Dacke 2010). Finally, it is the tapetum of a lycosoid that accounts for eyeshine, the bright pinpoints of glittering reflection experienced by a person wearing a headlamp that is pointed at the spider from a distance.

In the present study, we measured the intensity and spectral properties of eyeshine from the PMEs in lycosoid spiders in two families: Lycosidae (wolf spiders) and Pisauridae (nursery web spiders). Spiders in these families have much in common.

First, they share a common phylogeny, being on the same branch of the "higher lycosoids" with Miturgidae and Trechaleidae (Coddington 2005). In addition, and perhaps therefore, they also share many morphological (e.g., stance, shape), predatory (e.g., wander and pounce), circadian (crepuscular or nocturnal) and habitat characteristics. We might expect, then, that they would also share characters that enhance evening and night vision, and this expectation serves as our null hypothesis: because of their shared phylogeny, lycosids and pisaurids should vary little, either between families or within families, in the attributes of their eyeshine. On the other hand, a close look at each family reveals numerous differences (Ubick et al. 2005), both within and between the families, raising the possibility that eyeshine, too, would vary. Although lycosids are known to share grate-shaped tapeta, as do many other spider taxa, there are variations in the overall structure and function of the tapeta within this group (Land 1985). Certainly, many individual analyses of lycosoid eye structures have been conducted (Fenk & Schmid 2010; Jonasova & Kozmik 2008; Ortega-Escobar 2006; and see references therein), with a particular emphasis on *Cupiennius salei* (Strausfeld & Barth 1993). The degree and patterns of variations in eye morphology and function within and between the lycosoid spiders is not known. Variation, then, serves as our working hypothesis: because of divergent recent evolutionary histories, pisaurids and lycosids should be quite different in the attributes of their eyeshine, and those differences should be greater between the families than they are within the families.

METHODS

Spiders.—We concentrated on four species of fishing spiders (Araneae: Pisauridae) and four species of wolf spiders (Araneae: Lycosidae). The pisaurids, *Dolomedes tenebrosus* Hentz 1844, *D. triton* (Walckenaer 1837), *D. scriptus* Hentz 1845, and *D. vittatus* Walckenaer 1837, were collected from two sites in Bedford County, Virginia, in September and October 2011. The lycosids, *Gladicosa pulchra* (Keyserling 1877), *Hogna* sp. (cf. *Lycosa lenta* (Hentz 1844) sensu Wallace 1942), *Rabidosa punctulata* (Hentz 1844), and *Varacosa avara* (Keyserling 1877), were collected in Lafayette County, Mississippi, in September 2011. In all taxa, our subjects included only mature or penultimate females.

Captured spiders were transported and maintained either on water with closed-cell foam floats in 710-ml plastic deli containers (pisaurids) or in 44–147-ml vials (lycosids). During their brief maintenance period, ending on the day of experimentation 2–7 days after capture, the spiders were provided with water but no food.

On the day of experimentation, we used a random number generator both to determine the order of spider testing by species and to determine the order of testing of individuals within each species, ensuring that any order or time-of-day effects would be randomly distributed among and within the eight taxa of spiders.

Specimen preparation, mounting, and positioning.—Spiders were anesthetized with CO_2 and killed by the rapid (<15 s, total) amputation of the abdomen at the pedicel and of all eight legs at the coxa-trochanter joints. The cephalothorax was then glued, sternum down, using quick-setting epoxy glue (Loctite® Weld™) to a small platform of card stock that was itself attached with glue or tape to the top of a 3-mm diameter steel flat-topped screw. This screw with its appended cephalothorax was then mounted at the top of a planetary gear assembly that was driven by a computer-controlled stepping motor (Fig. 2). The cephalothorax/eard/screw combination made it possible to center the left posterior median eye (PME) approximately on the long axis of the screw, so that rotation of the screw would leave the PME at the same location (but with a different orientation). Our standardized position had the axis of rotation 21° from vertical, leaning toward the camera or the spectrometer probe (Fig. 2), with the specimen rotated 22.5° clockwise relative to the starting position at which both posterior median eyes were facing the camera or probe. The precision of this standardized 22.5° clockwise rotation was achieved using the computer-controlled planetary gear assembly. This put the camera or probe directly opposite and facing the left PME of each specimen. Representative resulting camera views are shown in Figs. 1A & B.

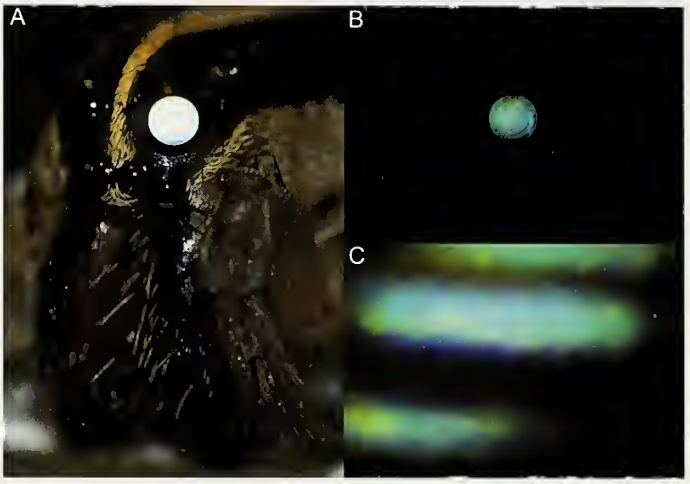

Figure 1.—Eyeshine of *Rabidosa punctulata*. A) Reflection from the tapetum of the posterior medial eye is visible because part of the lighting for the image was coaxial; i.e., in line with the lens-to-sensor axis of the camera. Indirect lighting was provided by an off-camera flash. B) In the absence of indirect lighting, and with a shorter exposure, only the tapetal reflection is visible and no other reflections (e.g., from other eyes or the spider's cuticle) can be seen. C) Under identical lighting conditions but with the lens removed from the camera and using a longer exposure, some of the structure of the tapetum itself is visible.

The assembly bearing the specimen and its positioning hardware was easily slid between two precisely marked locations on the laboratory bench. At one location, the specimen was directly in front of a camera (Nikon D200) attached to a photographic bellows (Nikon PB-4) that was equipped with an enlarging lens (50 mm f/3.5). Lighting to make eyeshine visible to the camera was provided by a high-output white LED (Radio Shack 276-0005) made coaxial with the camera-to-specimen axis by directing the light using a glass coverslip (thickness = 0.145 mm) as a partially silvered mirror (see Fig. 1 in Mueller & Labhart 2010). The photographic assembly, including the light source, was fixed in place so that when the specimen was in view, it was already in focus and coaxially illuminated.

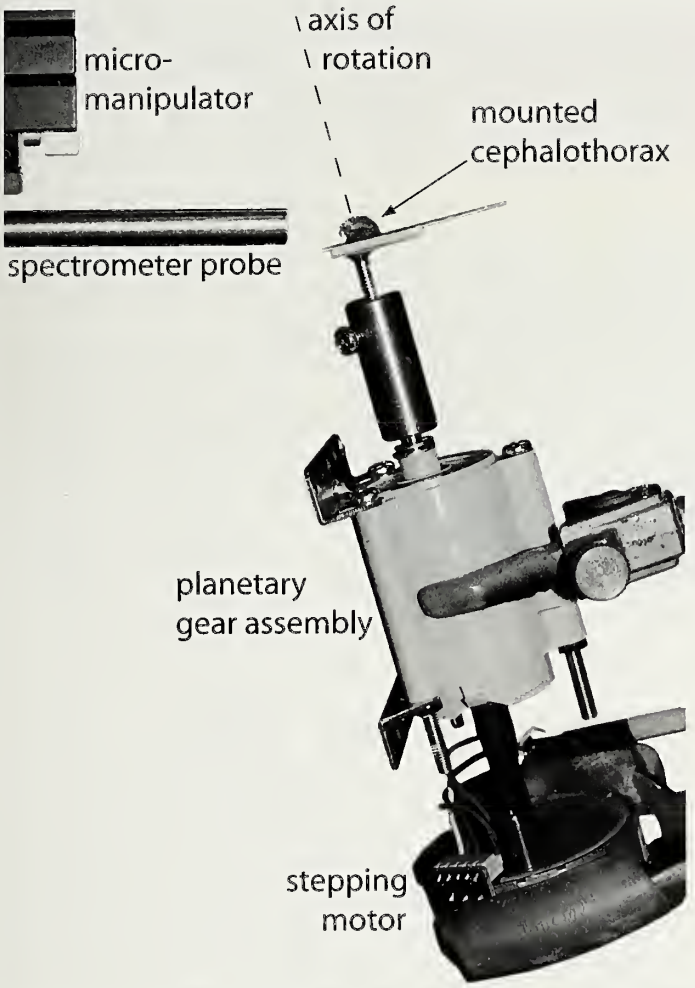

Figure 2.—Details of positioning of a spider's cephalothorax. The stepping motor drove a planetary gear assembly resulting in rotation of the specimen in increments of 0.09°. The angle of the axis of rotation was fixed at approximately 21° from vertical. The spectrometer probe was horizontal. It bore six optical fibers for illumination surrounding a single read fiber with a diameter of 400 mm and an acceptance angle of 24.8°.

When placed at the second location, the specimen was about 0.5 cm from the front face of the micromanipulator-mounted fiber optic probe used both to illuminate the left PME and to collect the reflected light for spectral analysis. The core of the probe (Ocean Optics QR400-7-UV-VIS) consisted of six illuminating optical fibers surrounding a single read optical fiber so that, as was the case with the camera's illumination, the illumination used for spectral collection was coaxial with the sensor-to-specimen axis. Each of the seven fibers in the probe had a diameter of 400 μm, and the read fiber had an acceptance angle of 24.8°. The measured intensity of light reflected by the spiders' tapeta varied with the distance between the face of the probe and the left PME. To normalize intensity so that comparable spectra could be collected, we used the micromanipulator to adjust the probe-to-PME distance so that the peak intensity recorded by the spectrometer was close to 10,000 counts (measured mean ± SE: 9,785 ± 116). We then measured the probe-to-PME distance as a direct index of reflection intensity—the closer the probe had to be to achieve 10,000 counts, the dimmer the reflection was.

Experimental procedures.—We used two procedures in this study, one to collect standardized data from female representatives of each of the eight species of spiders and the other to collect longitudinal post-mortem data from one spider. For both procedures, the preparatory stages were identical (above).

Our standardized data collection procedure involved making three photographic exposures, shifting the specimen-positioning assembly 25 cm to the location of the spectrometer probe, and collecting one spectrum. The three photographs, in order, were 1) an image of the spider illuminated both by the coaxial LED light and an overhead flash (exposure: 1/16 of maximum flash intensity, f/8, 1/10 s, with sensor set at ISO 400); 2) an image of the spider illuminated only by the coaxial LED light (exposure: 1/100 s exposure f/8 with sensor set at ISO 400); and 3) an image from exactly the same position but with the enlarging lens removed so that no optics intruded between the spider's eye and the camera's sensor (exposure: 1 s with sensor set at ISO 400). In the lens-on conditions (1 & 2), the enlarging lens's surface was 61 mm from the spider's PME; in the lens-off condition (3), the sensor was 214 mm from the PME.

We collected the single spectrum after adjusting the probe-to-eye distance, as described above. Spectra were automatically time-stamped, making it possible to determine how long it took to run a specimen through the standardized procedure. It took 7.0 ± 0.3 min (mean ± SE) for two of us to position, photograph and collect a spectrum from one spider. We anaesthetized, killed, and mounted the spiders one at a time, in the same order in which they were to be tested; we estimate that each took about four min to prepare once anesthesia was achieved. As a result, a spider's maximum time from death to the end of the measurement procedure was about 11 min.

We undertook the collection of longitudinal data, spectra only, on a single female *Hogna* sp. The purpose of this procedure was to get an estimate the rate at which tapetal reflection decayed after death, our concern being to avoid tapetal degradation as a confounding variable. In this procedure, we anesthetized, killed, mounted, and positioned the spider in our standardized way, skipped the photography, and collected 22 spectra over the subsequent five h.

Spectral measurement and analysis.—Light produced by a tungsten halogen light source (Ocean Optics HL-2000; color temperature = 2,960 K) was delivered to the specimen via optical fiber, and the part of it reflected by the left PME was transmitted via optical fiber to a high-resolution spectrometer (Ocean Optics 4000). Output from the spectrometer was collected by software (SpectraSuite by Ocean Optics) and exported as text (3,648 wavelength-intensity pairs; wavelength range = 357.9–819.5 nm). When spectra were collected, the only other light sources in the laboratory were the fluorescent fixtures providing general room illumination. A light meter, with its sensor positioned at the location and orientation of a spider's left PME during testing, revealed that the fiber optic illumination was 225–5892 times as intense (varying inversely with the distance between the fiber optic source and the light sensor) as the general room illumination at that same location. This, as well as inspection of spectra when the room lights were on and when they were off, persuaded us that making our

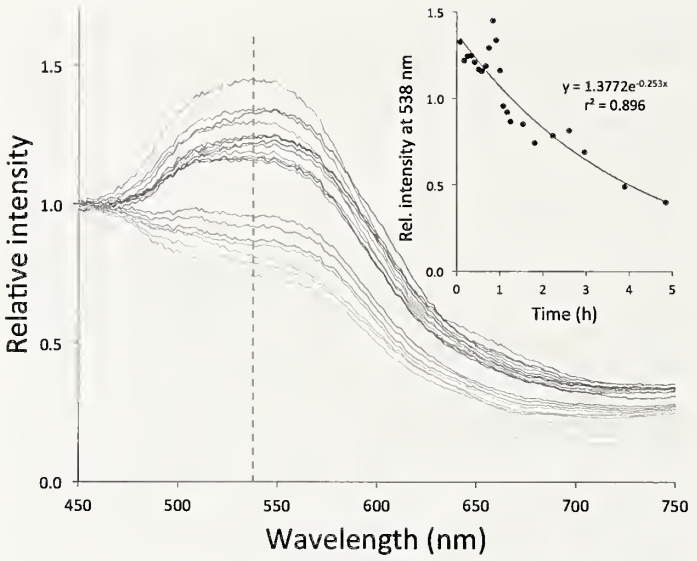

Figure 3.—Reflectance spectra showing the decline in eyeshine intensity with time after the death of a single *Hogna* sp. Spectra taken earlier in the post-mortem period are rendered with darker lines. The vertical dashed line is set at 538 nm. Inset: the intensity of eyeshine at 538 nm as a function of time after death; the line shows exponential decay of intensity.

spectral measurements under general room illumination would not influence our results.

We used a Mathematica 8 (Wolfram Research, Inc.) procedure to make a derivative spectrum composed of the 364 blocks of 10 intensity measures (averaged) in the original spectrum. We repeated this data reduction and smoothing step for all PME reflection spectra and for a spectrum that measured the tungsten halogen light source itself. In Excel (Microsoft Excel for Mac 2011), we divided each PME spectrum intensity value by the intensity value of the illuminating light source at the corresponding wavelength, resulting in the relative intensity spectra that we subsequently used in all of our analyses.

We adopted peak wavelength (the wavelength at which a spectrum had the highest relative intensity) as our metric of the characteristic shape of a spectrum, comparing peak wavelengths among species via ANOVA and Tukey's multiple comparison tests, and between families using Student's t-test. But characterizing a spectrum with one number could be so crude an abstraction that other salient characteristics could be missed. Therefore, to explore other kinds of differences in the spectra at the species and family level, we looked at the slopes of 15 30-nm segments of the spectra (sensu Thorpe 2002) using R (http://www.R-project.org). These segments included each 30-nm segment from 360–810 nm. These 15 data points for each spider were analyzed using principal components analysis (PCA), where PCA then generates 15 independent components, each a linear combination of the 15 slope values. The first of these components was then analyzed by ANOVA, just as we had analyzed peak wavelengths. We calculated the linear correlation between peak wavelengths and the corresponding first components derived from the PCA procedure to determine whether the PCA was revealing salient features of the spectra that had been overlooked because of our reliance on peak wavelengths.

Table 1.—ANOVA of the peak eyeshine wavelengths (Fig. 4A) of the eight species of lycosoids. Overall, the variation was highly significant ($F_{7,53} = 8.897$, $P < 0.0001$). When the data were pooled by family (Fig. 4A), the mean wavelength of the pisaurids was significantly longer by 20 nm (one-tailed $t_{59} = 2.87$, $P = 0.0033$). Only the significant comparisons ($P < 0.05$) are shown for the ANOVA post hoc tests.

Variance	Sum of squares	df	Proportion of variance
Between species	27670	7	0.54
Within species	23540	53	0.46
Tukey's Multiple Comparison Test	**Mean difference (nm)**	***q***	***P***
D. triton vs. *V. avara*	36	4.69	< 0.05
D. vittatus vs. *Hogna* sp.	38	5.67	< 0.01
D. vittatus vs. *R. punctulata*	40	5.93	< 0.01
D. vittatus vs. *V. avara*	51	7.37	< 0.001
G. pulchra vs. *Hogna* sp.	54	7.14	< 0.001
G. pulchra vs. *R. punctulata*	55	7.37	< 0.001
G. pulchra vs. *V. avara*	67	8.65	< 0.001

Morphological measurements.—After testing, specimens were preserved in 95% ethanol. We measured left PME diameters using ImageJ (freeware from the National Institutes of Health) to analyze the photographs of eyeshine in which illumination came both from an overhead flash and from the coaxial LED light source (e.g., Fig. 1A). We measured prosoma width, a commonly-used index of overall spider size (Hagstrum 1971; cf. Suter & Stratton 2011), with an ocular micrometer while viewing each ethanol-preserved specimen under a dissecting microscope.

RESULTS

Post mortem, the eyeshine in *Hogna* sp. decayed exponentially over the course of five h (Fig. 3), as measured by the intensity of reflected light at 538 nm. The regression equation in Fig. 3 allowed us to calculate the expected change in eyeshine intensity at 11 min, the maximum time between death and our collection of the reflectance spectrum and the peak intensity measurement for any spider we tested. At 11 min post mortem, the eyeshine would have decayed by 4.1%. For our purposes, this indicates that collecting data within the first 11 min post mortem confined the temporal component of intensity and spectrum variation to less than 5%. In addition it is worth noting that Fig. 3 shows hints of complexity in the decay of eyeshine (the rise above baseline values as the 1-h mark approached).

Spectral characteristics.—Peak eyeshine wavelengths varied significantly (Table 1, Fig. 4A) both among the eight species tested ($F_{7,53} = 8.897$, $P < 0.0001$) and between Pisauridae and Lycosidae (one-tailed $t_{59} = 2.87$, $P = 0.0033$). On average, the pisaurid spectra peaked within the green part of the spectrum, but about 20 nm more toward yellow than did the lycosid spectra. The variation among species (about 54% of the total variation in peak wavelength) was attributable especially to *G. pulchra* (Lycosidae) and *D. vittatus* (Pisauridae), both of whose spectra peaked in or near yellow, vs. *V. avara* (Lycosidae), whose spectra peaked to the blue side of green. The mean peak

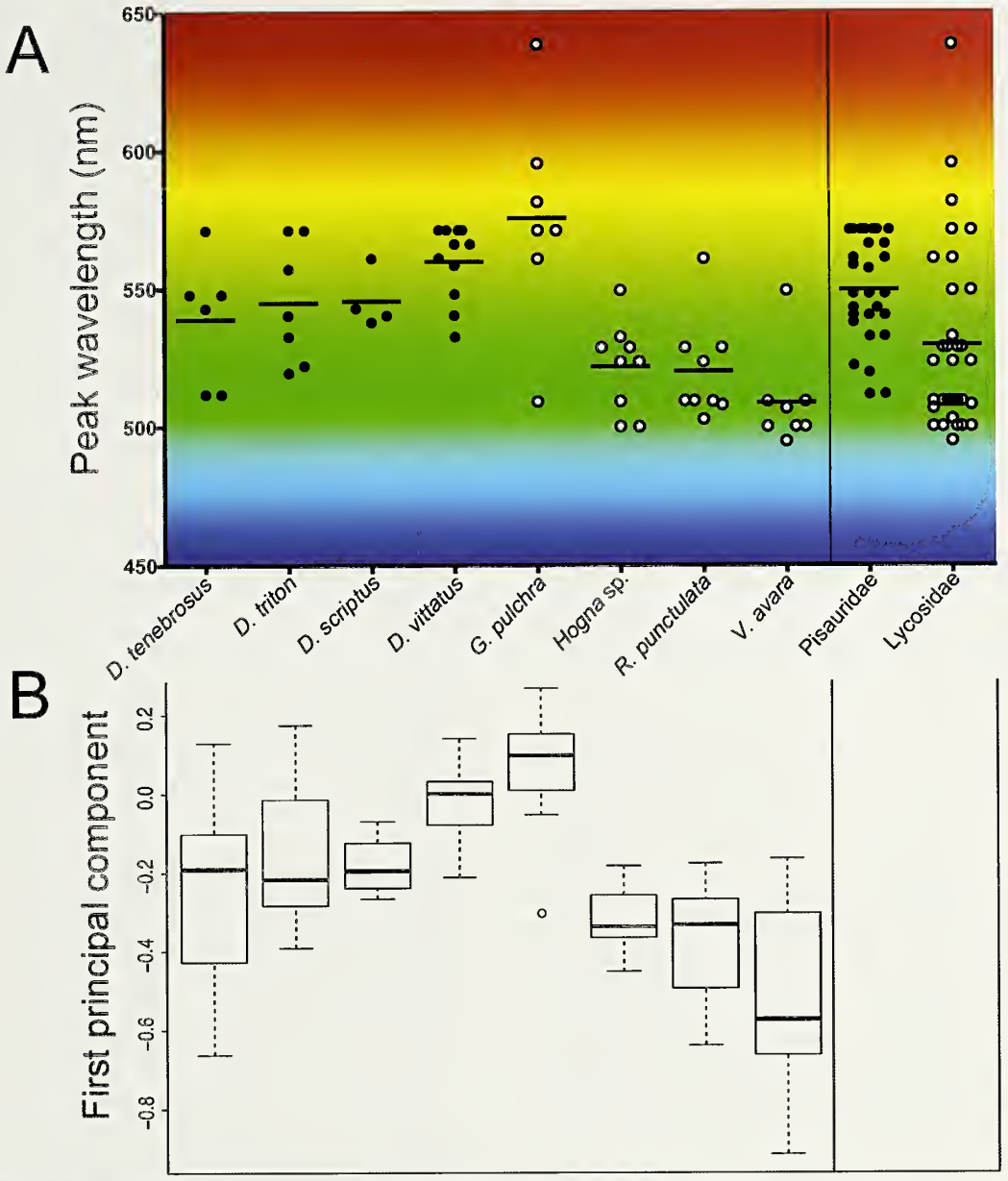

Figure 4.—A) Mean peak eyeshine wavelengths varied from yellow (e.g., *Gladicosa*) to blue-green (e.g., *Varacosa*). The mean wavelength of the eyeshine of the pooled pisaurids was more toward the red than was the mean of the pooled lycosids. Analyses of these differences are shown in Table 1. B) Principal component analysis yielded a first component that closely matched the peak wavelength data, indicating that most of the variation in spectral properties was captured by our analysis of the peak wavelengths. In this plot, heavy horizontal lines represent means, the boxes show 95% confidence intervals, and the whiskers indicate ranges; the single open circle for *Gladicosa* designates an outlier.

wavelength of *G. pulchra* (yellow) and that of *V. avara* (blue-green) were separated by 67 nm.

Our principal components analysis of the spectra, designed to reveal differences that were not captured by our use of peak wavelength as an index of overall spectral shape, failed to elucidate any additional salient spectral characteristics. The first principal components closely matched the peak wavelengths (Fig. 4B; $r = 0.969$, $P < 0.0001$). For this reason, we adopted peak wavelength as our sole index of the spectral quality of tapetum reflectance.

Eyeshine intensity.—The intensity of a spider's eyeshine depends not only on how much of the light entering the eye leaves the eye again as reflected light but also, presumably, on the size of the eye itself. Our interest was in the former, so we had to eliminate eye size as a confounding variable. PME diameter varied linearly with prosoma width (our proxy for spider size) in both lycosids and pisaurids (Fig. 5), with lycosid eye diameters exceeding pisaurid eye diameters by 65–85% in the range of overlap of prosoma widths. PME diameter also varied significantly (Fig. 6, Table 2) among the eight species studied ($F_{7,62} = 27.02$, $P < 0.0001$) and between the pooled Pisauridae and the pooled Lycosidae (two-tailed $t_{68} = 4.75$, $P < 0.0001$). Our solution was to regress measured eyeshine intensity, as measured by the distance between the spectrometer probe and the spider's PME, on PME diameter—eyeshine intensities above the regression line would then represent brighter eyeshine than would be expected relative to eye diameter, and intensities

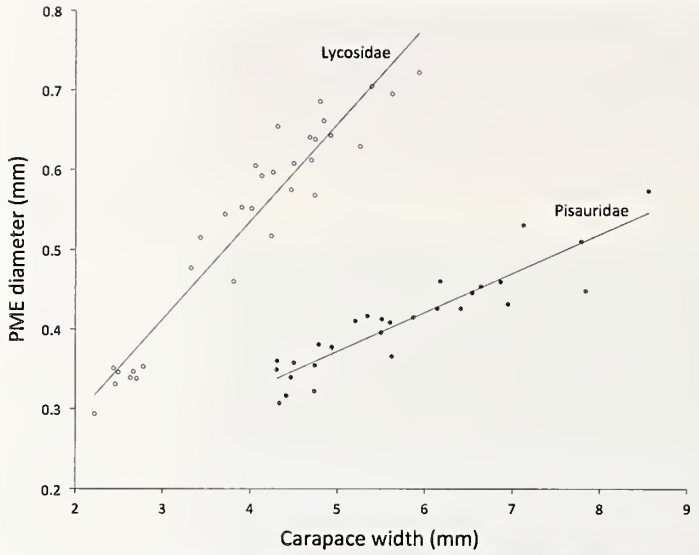

Figure 5.—In both Lycosidae and Pisauridae, PME diameter varied linearly with carapace width (Lycosidae: $r^2 = 0.918$, $P < 0.0001$; Pisauridae: $r^2 = 0.839$, $P < 0.0001$). The slopes of the lines were significantly different ($F_{1,56} = 87.28$, $P < 0.0001$). In the range of overlap of carapace widths, lycosid PME diameters are 65–85% larger than pisaurid PME diameters.

below the regression line would represent relatively dim eyeshine.

As expected, eyeshine intensity did vary directly with PME diameter (Fig. 7), and variation in eye size accounted for about 42% of the variation in eyeshine intensity ($r^2 = 0.4193$, $F_{1,45} = 32.49$, $P < 0.0001$). Residuals from this regression relationship (Fig. 8, Table 3) showed significant variation overall (ANOVA, $F_{7,39} = 4.661$, $P = 0.0007$), and pisaurid eyeshine was significantly brighter, relative to eye size, than lycosid eyeshine (two-tailed $t_{45} = 3.64$, $P = 0.0007$). *V. avara*, the lycosid spider with the smallest eyes of all the species (Fig. 6), also had the dimmest eyes relative to eye diameter (Fig. 8) and accounted for most of the between-species variation in relative brightness of eyeshine (Table 3).

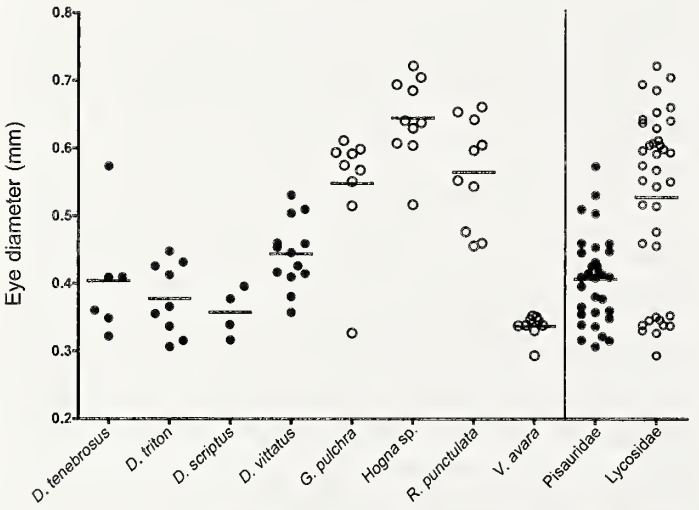

Figure 6.—PME diameter varied strongly among the eight species of spiders and between the two families. Lycosids had larger PMEs than did pisaurids, and lycosid eyes were also more variable (Table 2).

Table 2.—ANOVA of posterior median eye diameters (Fig. 6) of the eight species in the study. Overall, the variation was highly significant ($F_{7,62} = 27.02$, $P < 0.0001$). When the data were pooled by family (Fig. 6), the eyes of the lycosids were significantly larger by 0.13 mm (two-tailed $t_{68} = 4.75$, $P < 0.0001$). Only the significant comparisons ($P < 0.05$) are shown for the ANOVA post hoc tests.

Variance	Sum of squares	df	Proportion of variance
Between species	0.7698	7	0.75
Within species	0.2523	62	0.25
Tukey's Multiple Comparison Test	Mean difference (mm)	*q*	*P*
D. tenebrosus vs. *G. pulchra*	−0.14	6.06	< 0.01
D. tenebrosus vs. *Hogna sp.*	−0.24	10.33	< 0.001
D. tenebrosus vs. *R. punctulata*	−0.16	6.91	< 0.001
D. triton vs. *G. pulchra*	−0.17	8.01	< 0.001
D. triton vs. *Hogna sp.*	−0.27	12.88	< 0.001
D. triton vs. *R. punctulata*	−0.19	9.03	< 0.001
D. vittatus vs. *G. pulchra*	−0.10	5.33	< 0.01
D. vittatus vs. *Hogna sp.*	−0.20	10.58	< 0.001
D. vittatus vs. *R. punctulata*	−0.12	6.38	< 0.001
D. vittatus vs. *V. avara*	0.11	5.44	< 0.01
D. scriptus vs. *G. pulchra*	−0.19	7.03	< 0.001
D. scriptus vs. *Hogna sp.*	−0.29	10.76	< 0.001
D. scriptus vs. *R. punctulata*	−0.21	7.77	< 0.001
G. pulchra vs. *Hogna* sp.	−0.10	4.66	< 0.05
G. pulchra vs. *V. avara*	0.21	9.90	< 0.001
Hogna sp. vs. *V. avara*	0.31	14.82	< 0.001
R. punctulata vs. *V. avara*	0.23	10.97	< 0.001

The differences in eyeshine we have reported here require that we reject our null hypothesis that, because of their shared phylogeny, lycosids and pisaurids should vary little, either between families or within families, in the attributes of their eyeshine. Instead, we accept the general assertion that, because of divergent recent evolutionary histories, pisaurids and lycosids and their constituent species show considerable variation in the attributes of their eyeshine.

DISCUSSION

Our sampling of eyeshine from eight species in two families of lycosoid spiders revealed a surprising and complex array of differences. The two families, Pisauridae and Lycosidae, had mean peak reflectances that differed significantly (Fig. 4). The intensity of eyeshine was strongly influenced by eye diameter (Fig. 7), and lycosids had larger eyes relative to body size than did pisaurids. However, residuals from the regression of reflection intensity on eye diameter showed that pisaurid eyeshine was significantly brighter (relative to eye size) than lycosid eyeshine (Fig. 8). In addition to those strong family differences, we also detected interesting species-level variation in peak reflectance and eyeshine intensity. Peak reflectance was relatively uniform among the pisaurids but among the

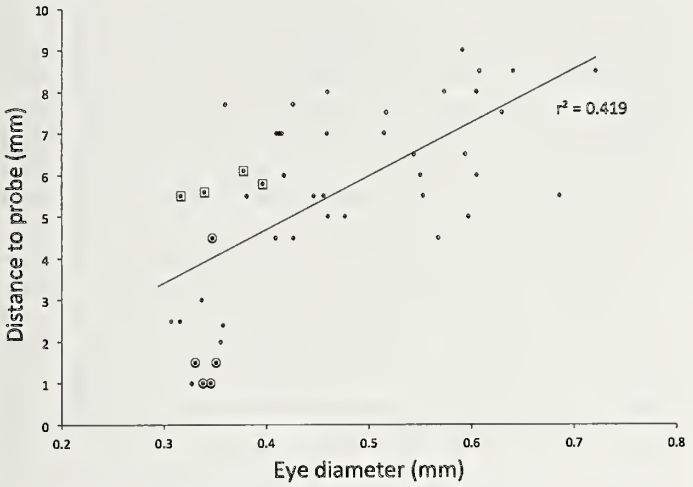

Figure 7.—Eye diameter appears to drive the intensity of eyeshine, accounting for about 42% of the variation in intensity. The most conspicuous variant was *V. avara* (circled data) with eyeshine that was significantly dimmer than expected relative to eye diameter. In contrast, *D. scriptus* (data surrounded by squares), a spider with eyes of about the same size, had eyeshine that was much brighter relative to eye diameter (Fig. 8).

lycosids, *Gladicosa pulchra* was significantly red-shifted relative to the other three species (Fig. 4). And with respect to intensity, *D. scriptus, D. tenebrosus,* and *D. vittatus* were brighter (relative to eye size) than expected and *V. avara* was dimmer (Fig. 8).

Spectral composition of eyeshine.—As expected (Schwab 2002), the peak reflectivity of the eyeshine of both pisaurids and lycosids was in the green range, but the average peak lycosid eyeshine was significantly more toward blue-green than was the peak for pisaurids. However, family of origin accounted for less spectral peak variation than did species identity (Table 1), and the primary source of species variation

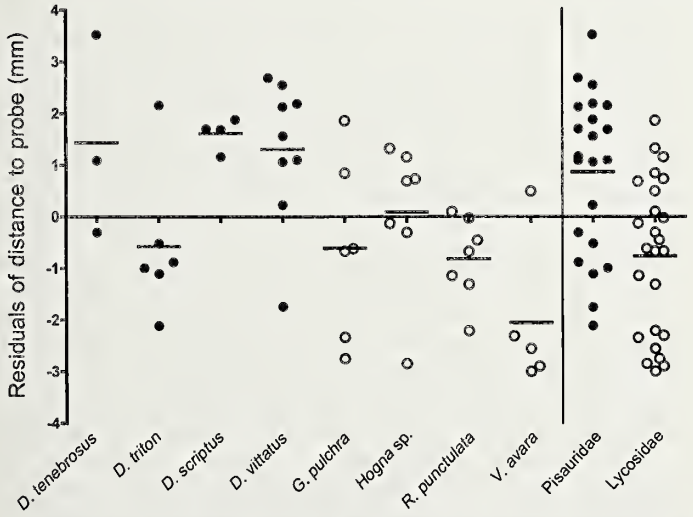

Figure 8.—Residuals from the regression of eyeshine intensity on PME diameter (Fig. 7) show that pisaurids' eyes, though smaller, are brighter relative to their size than are the larger eyes of lycosids (Table 3). *Varacosa avara*, a wolf spider, had the dimmest eyeshine relative to PME diameter, while *D. scriptus*, a fishing spider, had the brightest.

Table 3.—ANOVA of the residuals of the regression of eyeshine intensity on PME diameters (Fig. 8) for the eight species studied. Overall, the variation was highly significant ($F_{7,39} = 4.661$, $P = 0.0007$). When the data were pooled by family (Fig. 8), the residuals of the pisaurids were significantly higher by 1.63 mm (two-tailed $t_{45} = 3.64$, $P = 0.0007$), indicating that the pisaurids' eyeshine was significantly more intense relative to the spider's eye diameters. Only the significant comparisons ($P < 0.05$) are shown for the ANOVA post hoc tests.

Variance	Sum of squares	df	Proportion of variance
Between species	61.81	7	0.46
Within species	73.89	39	0.54
Tukey's Multiple Comparison Test	Mean difference (mm)	q	P
D. tenebrosus vs. *V. avara*	3.489	4.91	< 0.05
D. vittatus vs. *V. avara*	3.353	6.18	< 0.01
D. scriptus vs. *V. avara*	3.658	5.60	< 0.01

was the significantly red-shifted reflectance spectrum of the lycosid, *G. pulchra* (Fig. 4). Less conspicuous as sources of variation were the spectra of two of the pisaurids, *D. vittatus* and *D. triton,* that showed peaks at significantly longer wavelengths than did some of the lycosids. Selection or phenotypic plasticity related to behavioral and sensory attributes at the species level may account for these differences.

Outside of arachnids, closely related organisms in several taxa have been found to show differences in peak reception. These differences may correspond to the spectral properties of the environment. In several aquatic taxa, where the spectral environment varies strongly with depth, reception has been shown to be tuned to habitat. For example, moray eels' retinal structures, pigments, and spectral responses were consistent with the spectra available at the native depths of particular species (Wang et al. 2011). Seabream visual pigments vary such that the neural response is tuned to the environment (Wang et al. 2009). In stomatopods, midband receptors were tuned to the spectral environment (Cronin et al. 2000). These differences can be plastic. Differences in the spectral environment can result in developmental shifts in photoreceptor pigments, receptor morphology, and/or filtering pigments (guinea pigs, Hu et al. 2011; stomatopods, Marshall et al. 2007; and cichlid fish, Wagner & Kröger 2005). Evolutionarily, Fleishman (1992) reports feedback between sensory systems and signaling in lizards, suggesting coevolution. Similarly, despite likely costs due to avian predation, male lepidoptera produce colors that link closely with peak color reception in females (Stavenga & Arikawa 2006). Spiders face similar constraints; salticid males lacking critical wavelengths of colors failed to elicit courtship from otherwise receptive females (Lim et al. 2008).

We measured reflectance rather than reception, but a similar phenomenon has been reported in deep-sea fishes (Douglas et al. 1998). Like spiders, these deep-sea fishes possess guanine-based tapeta, and have lenses that differentially filter light. Although spider and fish eyes differ in many ways, Douglas et al. (1998) report that eyeshine is tuned to the spectral

environment of the fishes, is influenced both by tapetal reflection and ocular media, and is a relevant measure of sensitivity in fishes. Similarly, peak reflectance may relate to differences in the perceptive frequencies between the different spiders (Yamashita 1985). Although diel variation in the structure and sensitivity of the photoreceptors is known in spiders (Blest & Day 1978), the tapetum is not variable on a diel basis (Grusch et al. 1997). Differences in microhabitat use, positioning, or diel patterns of behavior could influence the quality of light experienced and thus optimal reception or reflection spectra. Considering the species in this study: the pisaurids are semi-aquatic (Carico 1973), *G. pulchra* is often arboreal (Eubanks & Miller 1992), and the other lycosids are typically found in disturbed soil, particularly in riparian zones (*Hogna* sp.: Walker et al. 1999), on leaf litter (*Varacosa* sp.: Brushwein et al. 1992), or in grasses (*Rabidosa* sp.: Reed et al. 2008).

Habitat preferences have further behavioral and functional correlates that may also have influenced eye function and morphology. For example, there are notable differences in the typical posture and position of the spiders: *G. pulchra* orient vertically (downward) on trees (Eubanks & Miller 1992), the other lycosids are typically found in grasses or on leaf litter and may be best characterized as indifferent with respect to the direction of gravity, and the pisaurids orient at a downward angle, but not vertical, relative to the water surface (Carico 1973).

Relative intensity of the eyeshine.—Raw variation between species with respect to the intensity of reflection is partly a consequence of eye size. In our study, intensity varied linearly with eye diameter, and eye size accounted for about 42% of the variation in intensity (Fig. 7). At the same time, PME size in the spiders we studied varied substantially, with the lycosids, as expected, having larger eye diameters than did the pisaurids (Fig. 6, Table 2). Note that one of our initial observations had been that lycosid PME eyeshine was brighter than pisaurid PME eyeshine. This is strictly true, but only because, relative to spider size, lycosid PMEs are much larger (Fig. 5). Our analysis of the residuals from the regression of intensity on eye diameter provided a means whereby we could normalize intensity relative to eye diameter (Fig. 8, Table 3). Relative intensity of the eyeshine was quite variable, with the pooled pisaurids having brighter eyes relative to their eye diameters than the eyes of lycosids. The most conspicuous variants were the lycosid, *V. avara*, with eyeshine that was conspicuously dim relative to similarly sized PMEs to *D. scriptus*, which showed conspicuously bright eyeshine (Figs. 7 & 8). This is consistent with the overall trend of lycosid eyes reflecting less light relative to eye-size than the pisaurids.

At the family level, there was a strong and consistent difference in eye size and, correspondingly, overall intensity of reflection. In other taxa, Leuckart's Law suggests that faster moving animals would have larger eyes to maximize acuity, and such a correlation has recently been reported in mammals (Heard-Booth & Kirk 2012). In spiders, eye size is thought to correlate to visual acuity and, in general, eye size has been used as a proxy for the importance of visual stimuli (Pirhofer-Walzl et al. 2007). Further, in the pisaurids, visual acuity has been shown to be useful in predator detection (Williams 1979), but predator and prey detection using other sensory modes has been demonstrated (Bleckmann & Rovner 1984; Bleckmann & Lotz 1987; Suter & Gruenwald 2000; Suter 2003). Similarly, other modes of communication are thought to be of primary importance for courtship and mating in pisaurid spiders (Roland & Rovner 1983; Arnqvist 1992). In the lycosids, larger eyes with greater light sensitivity would facilitate visual detection of predators and prey, and visual detection has been reported (Lohrey et al. 2009; Clemente et al. 2010). Substantial literature supports the importanee of multi-modal communication for sexual selection in lycosids, particularly including visual stimuli (Hebets 2005; Rypstra et al. 2009). Rovner (1996) reported that although vibrations enhanced mate-searching, visual signals were important courtship cues. One model suggests that the sensory apparatus of female lycosids coevolved with the elaborate signals (Hebets & Uetz 1999), and this model is supported by data suggesting that the vibratory component of courtship is aneestral and the visual component derived within the family (Stratton 2005; Taylor et al. 2007).

It is plausible that these size and reflective intensity differences are functionally linked to vision capabilities. All of the taxa examined are considered to be primarily nocturnal or crepuscular with activity patterns consistent with those measured in *Cupiennius* sp. (Schmitt et al. 1990; Pirhofer-Walzl et al. 2007). Increased reflection may increase visual responsiveness in low light situations, but likely at the cost of some visual acuity (Land & Nilsson 2001). Thus, we might find that the increased reliance on visual signaling is associated with reductions of refleetanee in lyeosid eyes (relative to the pisaurids) to afford increased acuity to the lyeosids. The conspicuously dim reflectance of the PM eyes in the small lycosid, *V. avara,* is consistent with such a trade-off. *Varacosa avara* has eyes similar in size to those of the much larger pisaurid spiders, but *V. avara's* eyeshine is much less intense than that of the pisaurids. Their relatively small size may limit eye size, and thus acuity, perhaps requiring reduced tapetal reflections to achieve sufficient acuity. Trade-offs between visual abilities and tapetal reflection have been shown elsewhere. For example, in a study of lampreys, only the burrowing species show increased tapetal reflection. In that situation, the tapetal reflection correlated to a loss of cones and, hence, color vision (Collin & Potter 2000). Similarly, in decapod shrimp, larger shrimp had tapeta producing more intense reflection; and these inereases were correlated to increased depth (and darker habitats) for these shrimp (Johnson et al. 2000). Selection favoring increased reflection at the cost of acuity in the *Dolomedes* species could be linked to these spiders' preferred microhabitat in riparian zones where light is often limited (Carico 1973), or may simply be consistent with less reliance on visual signaling.

It is important not to exaggerate the functional significance our results. The reflectance that we have measured as eyeshine has both spectral and intensity properties, but we are still ignorant about the functional meanings of those properties. It is not clear, for example, whether a shift in peak wavelength of 55–60 nanometers toward the orange part of the reflected spectrum confers on *Gladicosa pulchra* a functionally different capacity to detect certain colors. The light reflected from the secondary eyes has traversed several layers of tissue, each of which must modify parts of the spectra to some degree. For

example, in *Cupiennius salei*, the light passes the lens, the vitreous body, the hypodermis layer, and the sensory cells before the tapetum reflects some of the light back through the same materials. Because the reflected light we collected and analyzed had been filtered by several additional layers of tissue after its second pass through the sensory cells, it is not clear exactly what wavelengths were available to the sensory cells (from Fig. 1B in Grusch et al. 1997). With respect to intensity, we cannot yet know whether eyeshine that is brighter relative to eye diameter means that the eye is more efficient at collecting photons (because the tapetum is a more efficient reflector) or is less efficient (because a greater proportion of the light is reflected back into the environment). Further exploration on lycosoid tapetal structures may reveal patterns in the observed tapetal variation. Tapeta vary even within the Lycosidae, such that the grate structure that is apparent in the taxa included in this analysis is not apparent in all lycosids. In the diurnal *Pirata,* for example, we found a punctuated sheet (isolated reflective segments) similar to those that Land reports in the thomisid *Tharpyna* sp. (Land 1985). We look forward to further explorations of eyeshine in lycosoid spiders that may reveal not only evolutionary and developmental constraints on visual reception in low-light situations, but also the functional consequences of differences in tapetal reflection.

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Variation among clutches in the response of spiders to prey nutrient content

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Abstract. The phenotype of animals is often determined by an interaction between genes and the environment. In spiders, recent work has shown that the nutritional composition of prey can have a large effect on the growth and reproduction of spiders. I tested whether the growth of juvenile spiderlings was affected by an interaction between the clutch and the diet on which they fed (i.e., high or low nutrient) in both a wandering (*Tigrosa helluo* (Walekenaer 1837)) and a web-building (*Pholcus phalangioides* Fuesslin 1775) spider. Diet was manipulated by feeding spiderlings similar quantities of food that varied in their nutritional eomposition. The results for both species followed the same pattern. Overall, spiderlings fed the high-nutrient prey were larger, both in terms of mass and body size. However, there was significant variation in effect size among clutches, with some clutches showing large effects of nutrients on growth and other clutches showing little or no effect of nutrients on growth. In both species, there were no differences among clutches in the final mass and size of individuals on the low nutrient treatment. The differences among clutches were due to differences in the mass and size of spiderlings on the high nutrient treatments. These results highlight the importance of incorporating a diverse range of clutches or genotypes in studies of spider nutrition to ensure that the results are generalizable and not biased by particular genotypes or clutches.

Keywords: Diet, *Pholcus phalangioides*, *Tigrosa helluo*

The quantity and nutrient content of food available in nature is a major factor affecting the survival, growth and reproduction of spiders (Wise 1993, 2006). In particular, the addition of lipid, protein, or a eombination of nutrients to prey ean affect the life history and behavior of a range of wandering and web-building spiders (Mayntz & Toft 2001; Jespersen & Toft 2003; Mayntz & Toft 2006; Wilder 2011). Arthropod prey can vary widely in their nutrient content in nature, and understanding how nutrients affect spider performance can better aid in predicting prey choice by spiders or the potential consequences of ehanges in prey communities for spider populations.

The clutch that a spider belongs to can also have significant effects on survival and growth (Jakob & Dingle 1990; Uhl et al. 2004). Spiders from some clutches grow faster or larger than those from other clutches due either to maternal or genetic effects on offspring growth (Jakob & Dingle 1990; Uhl et al. 2004). Clutch can also interact with food quantity to affect spider growth, resulting in some clutches showing a greater response to increases in food availability than others (Jakob & Dingle 1990; Balfour 2004; Uhl et al. 2004). For example, in pholcid spiders (Araneae: Pholcidae), body mass, development time and body size are heritable, and this heritability contributes to signifieant gene by environment interactions in the growth of spiderlings (Jakob & Dingle 1990; Uhl et al. 2004). However, it remains unknown whether clutch and prey nutrient content also interact to affect spider survival or growth. Testing for clutch by diet interactions is important because it affects the selection of animals for experiments. If there are significant interactions between clutch and diet, then care would need to be taken to include a diverse range of clutches or genotypes of spiders in experiments to ensure that the results are generalizable and not biased by particular genotypes or clutches.

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The purpose of this study was to test whether clutches of spiders differed in the effects of prey nutrient content on growth. Using a split-clutch design, I compared growth rates of several clutches of wandering and web-building spiders fed high or low nutrient prey (*Drosophila melanogaster* that had either been raised on standard or nutrient supplemented media; Mayntz & Toft 2001). This experiment was conducted using clutches from three females of the wandering spider *Tigrosa* (formerly *Hogna*) *helluo* (Walkenaer 1837) (Araneae: Lycosidae) and four females of the web-building spider *Pholcus phalangioides* Fuesslin 1775 (Araneae: Pholcidae). These species were not directly compared, but were used to test whether clutch effects and interactions of clutch and food quality occurred in spiders with different life history strategies. Significant differences in growth between clutches or qualitative differences in the way that clutches responded to diet treatments would indicate that clutch is an important factor to include in future studies of spider growth.

METHODS

Drosophila melanogaster were used as prey in these experiments. Diptera may be an important component in the diet of both wandering (e.g., Nyffeler & Benz 1988) and web-building spiders (e.g., Jmhasly & Nentwig 1995), and the nutritional content of Diptera can vary substantially in the field (Markow et al. 1999; Jaenike & Markow 2003). In the laboratory, the nutritional quality of individuals of a single species of *Drosophila* spp. can be manipulated through the composition of the media on which they are raised (Markow et al. 1999; Mayntz & Toft 2001; Jaenike & Markow 2003; Mayntz et al. 2003; Jespersen and Toft 2003; Mayntz et al. 2005; Mayntz and Toft 2006). For the purposes of this study, vestigial-winged *D. melanogaster* were raised on either potato flake medium (Ward's™ Instant *Drosophila* Medium) or potato flake medium supplemented with 40% dog food (Ol' Roy™ Dog Food; 21% protein) by mass. There were no differences in the dry mass or percent C of flies raised on these treatments, but flies reared on the dog-food-supplemented

Table 1.—Results of repeated measures analysis of variance for the effects of prey nutrient content (low or high) and clutch on the mass of juvenile *Tigrosa helluo* and *Pholcus phalangioides* over time.

	Tigrosa helluo				*Pholcus phalangiodes*			
Source of Variation	df	MS	F	P	df	MS	F	P
Treatment	1	163.3	25.8	< 0.001	1	12.9	9.2	< 0.001
Clutch	2	13.1	2.1	0.136	3	23.5	16.7	< 0.001
Treatment*Clutch	2	41.8	6.6	0.002	3	0.5	0.4	0.666
Error	53	6.3			61	1.4		
Time	3	1654.7	749.3	< 0.001	6	415.8	867.7	< 0.001
Time*Treatment	3	91.2	41.3	< 0.001	6	5.6	11.6	< 0.001
Time*Clutch	6	7.9	3.6	0.002	18	4.9	10.3	< 0.001
Time*Treatment*Clutch	6	19.4	8.8	< 0.001	18	1.3	2.7	0.031
Error	159	2.2			366	0.5		

media (hereafter "high nutrient flies") did have higher nitrogen, lower C:N, lower lipid, higher protein and lower lipid:protein than flies reared on the potato flake medium alone (hereafter "low nutrient flies"; data in Schmidt et al. 2012).

Growth in the wandering spider, *Tigrosa helluo*.—Adult female *Tigrosa helluo* were collected in and around the agricultural fields at the Miami University Ecology Research Center (Oxford, Butler County, Ohio). Individuals were maintained in plastic containers (11 cm diameter × 8 cm high) with 1 cm of a peat/soil mix and fed two juvenile crickets (*Acheta domesticus*) twice per week. All females were fed similarly to reduce potential maternal effects. Three females produced egg sacs, which are carried attached to the female's spinnerets until they hatch. After hatching, spiderlings reside on the abdomen of the female for one to two weeks and then disperse (i.e., leave the mother's abdomen). For this experiment, I collected 20 dispersed spiderlings from each of three clutches produced by different females. The mass of dispersed spiderlings was 1.21 ± .02 mg (mean ± 1 SE). For this and the following experiment, spiderlings were separated before cannibalism occurred. A split-clutch design was used, in which spiderlings from each clutch were alternately assigned to high (n = 10) and low (n = 10) diet treatment groups. Each spiderling was placed in an individual translucent plastic container (8 cm diameter × 5 cm high) with one cm of moist peat moss. Individuals were fed *ad libitum* twice weekly with either low or high nutrient flies and weighed to the nearest 0.1 mg once weekly. The experiment was terminated after three weeks when individuals became too large to be maintained on *D. melanogaster*. Although I did not record the timing of molting, all individuals molted at least twiee during the experiment. At the end of the experiment, all individuals were sacrificed by freezing, and I measured carapace width, which is typically used as a measure of body size for wandering spiders (Jakob et al. 1996), to the nearest 0.01 mm using an ocular micrometer.

Growth in the web-building spider, *Pholcus phalangioides*.—Adult female *Pholcus phalangioides* with egg sacs were collected from a free-living population in Pearson Hall at Miami University. I collected similarly sized females with similarly sized egg sacs to reduce potential maternal effects. Egg sacs were removed from four separate individuals and allowed to hatch in separate deli containers (11 cm diameter × 8 cm high). For this experiment, I collected 20 dispersed spiderlings from each of the clutches and alternately assigned them to treatment groups. Each spiderling was placed in an individual translucent plastic container (3 cm diameter × 9 cm high). Individuals were fed ad libitum twice weekly with either low or high nutrient flies and weighed to the nearest 0.1 mg once weekly. The experiment was terminated after seven weeks when individuals became too large to be maintained on *D. melanogaster*. All individuals molted at least three times during the experiment. At the end of the experiment, all individuals were sacrificed by freezing, and I measured the combined length of the tibia and patella of one of the first pairs of legs, which is typically used as a measure of body size for web-building spiders (Jakob et al. 1996), to the nearest 0.01 mm using an ocular micrometer. In *P. phalangiodes,* tibia-patella length is highly correlated with carapace width (Schafer et al. 2008).

Statistical analyses.—I conducted repeated measures analysis of variance to test the effects of prey nutrient treatment, clutch and their interaction on mass separately for *T. helluo* and *P. phalangioides*. For measures of final body size, I conducted a two-factor analysis of variance to test the effects of prey nutrient treatment, clutch and their interaction on carapace width for *T. helluo*. I conducted a separate two-factor ANOVA to test the effects of the treatment factors on tibia-patella length for *P. phalangioides*. I used Tukey post hoc tests on least squares means for all possible comparisons to test for differences between low and high nutrient treatments of each clutch and for differences among clutches in growth on low or high nutrient treatments. Post-hoc comparisons were considered significant if $P < 0.05$. There were some deviations from normality and homogeneity of variance, although at least half of the treatment combinations for each species followed these assumptions. All statistical analyses were conducted in SAS 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Overall, there were significant effects of prey nutrient content on the growth of both *T. helluo* and *P. phalangioides* (Table 1, Fig. 1). Spiders raised on the high nutrient diet were, on average, 50% heavier than individuals on the low nutrient diet within three weeks for *T. helluo* and seven weeks for *P. phalangioides* (Fig. 1). Survival to the end of the experimental period was high in both *T. helluo* (overall: 98%) and *P. phalangioides* (overall: 86%).

Growth in the wandering spider, *Tigrosa helluo*.—There was a significant interaction between time, diet treatment and

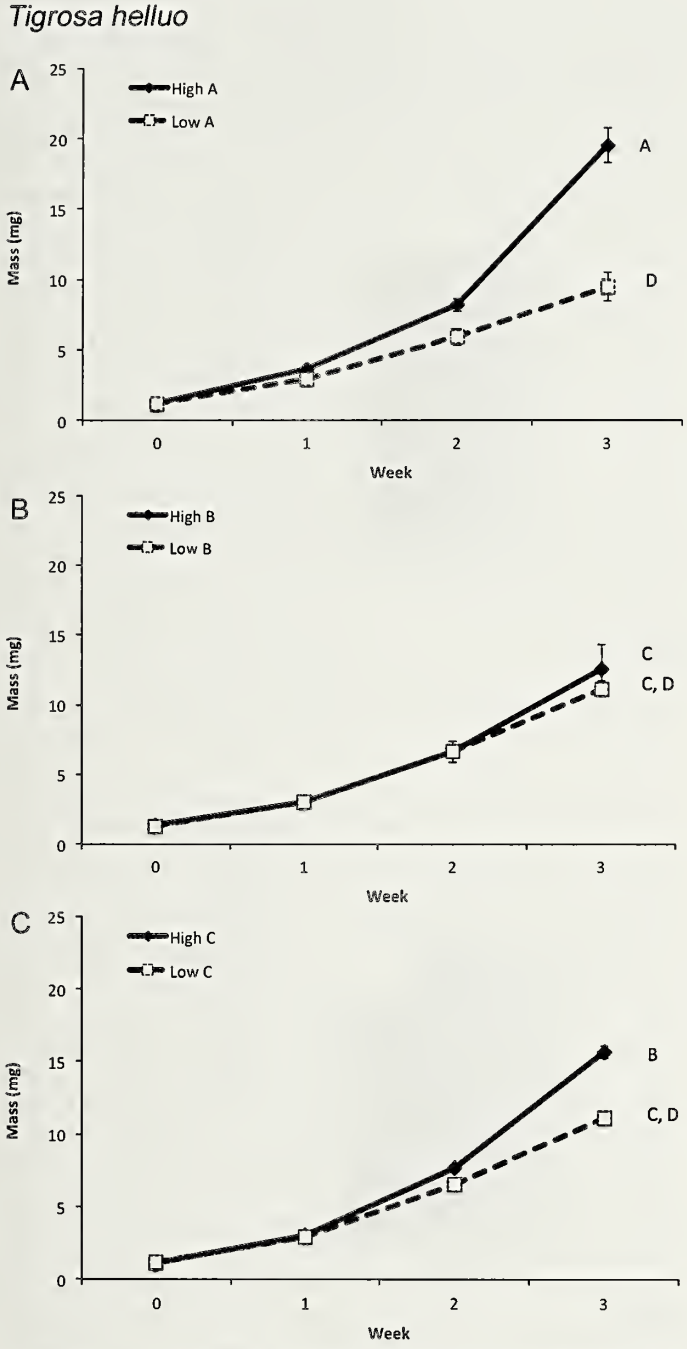

Figure 1.—Comparison of mean (± 1 SE) mass of juvenile *Tigrosa helluo* over three weeks on low or high nutrient diets from clutch A (A), clutch B (B), and clutch C (C). Post hoc comparisons were conducted for the final mass of spiders. Different letters show significant difference from each other at $\alpha = 0.05$.

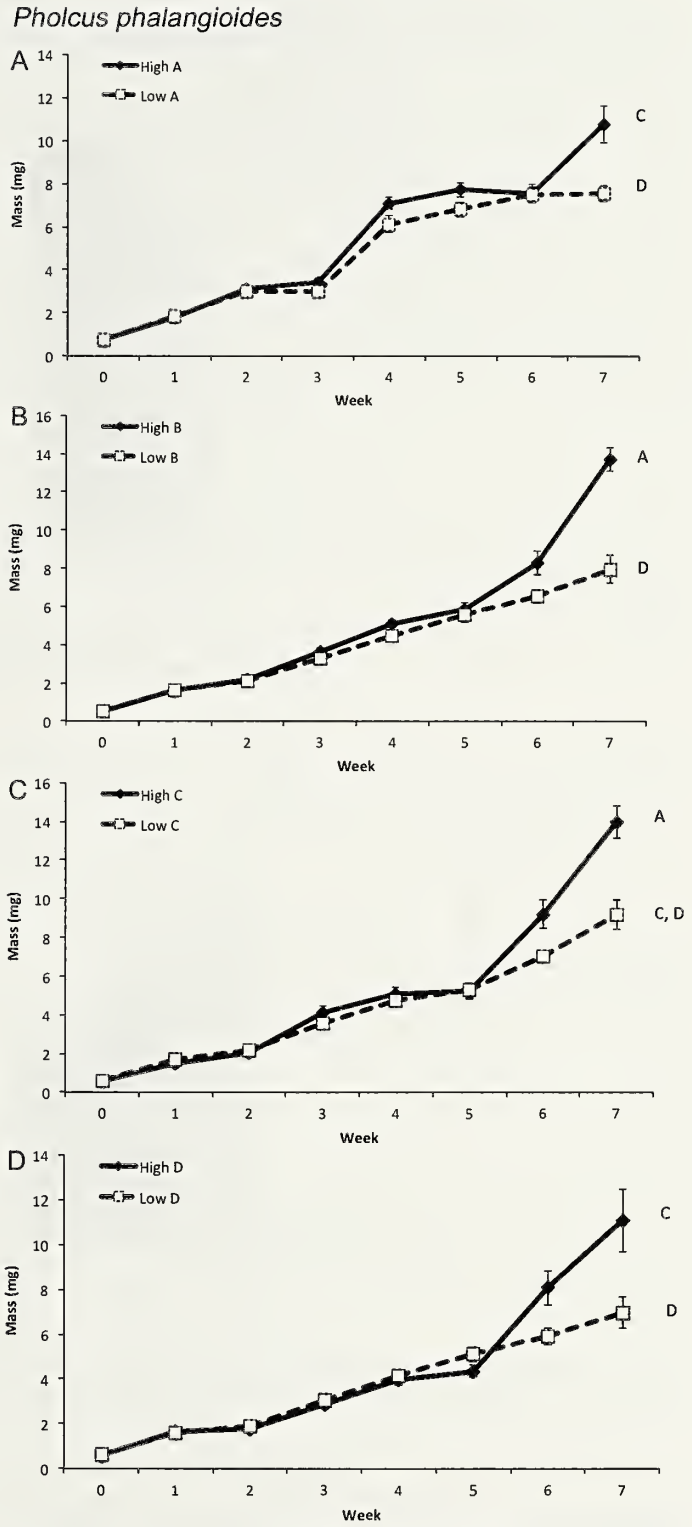

Figure 2.—Comparison of mean (± 1 SE) mass of juvenile *Pholcus phalangioides* over seven weeks on low or high nutrient diets from clutch A (A), clutch B (B), clutch C (C), clutch D (D). Post hoc comparisons were conducted for the final mass of spiders. Different letters show significant difference from each other at $\alpha = 0.05$.

clutch on the mass of juvenile *T. helluo*, indicating that the effects of prey nutrient content varied among clutches (Table 1, Fig. 1). Post hoc comparisons revealed significant differences between the mass of spiderlings on the low and high nutrient diets for clutches A and C, but no differences in the mass of spiderlings on the low and high nutrient diets for clutch B (Fig. 1).

There was a nonsignificant tendency for an interaction between clutch and diet on the final carapace width of *T. helluo* ($F_{2,48} = 3.02$, $P = 0.058$; Fig. 3A). As with mass, post hoc comparisons revealed that spiderlings from clutches A and C had significantly larger carapace widths on the high nutrient diet than on the low nutrient diet, but that there was no

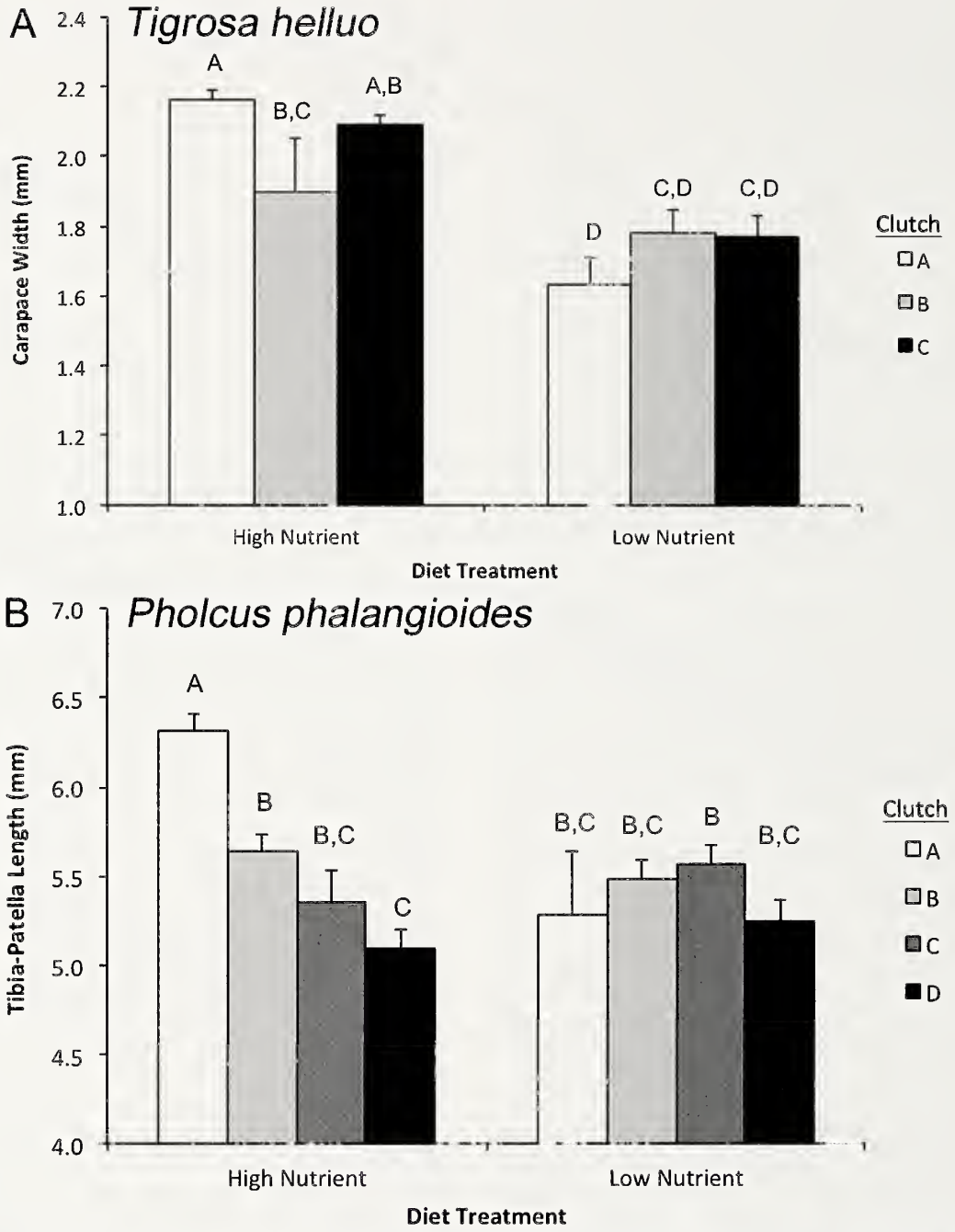

Figure 3.—Comparison of mean (± 1 SE) body size of A) *Tigrosa helluo* (measured as carapace width) and B) *Pholcus phalangioides* (measured as the length of the tibia and patella). Different letters show significant difference from each other at $\alpha = 0.05$.

significant effect of diet on the carapace width of clutch B (Fig. 3A).

Growth in the web-building spider, *Pholcus phalangioides*.— There was a significant interaction between time, diet treatment and clutch on the mass of juvenile *P. phalangioides*, indicating that the effects of prey nutrient content varied among clutches (Table 1, Fig. 1). In *P. phalangioides*, all four clutches showed higher mass on high nutrient diets (Fig. 2b). However, there were significant differences between clutches in the final mass of spiders on the high nutrient diet. Comparing spiderlings on the high nutrient diets, *P. phalangioides* from clutches B and C were significantly larger than those from clutches A and D (Fig. 2). There were no differences among clutches in the final mass of spiders on the low nutrient diet.

There was a significant interaction between diet treatment and clutch on the final tibia-patella length of *P. phalangioides* ($F_{3,59} = 5.59$, $P < 0.001$; Fig. 3B). Post hoc comparisons revealed a significantly larger tibia-patella length of spiderlings on the high nutrient diet than on the low nutrient diet for clutch A, but that clutches B, C, and D showed no significant effect of diet treatment on final tibia-patella length (Fig. 3B).

DISCUSSION

Many studies have demonstrated that the nutrient content of prey affects the survival and growth of spiders (Mayntz & Toft 2001; Mayntz et al. 2003; Jespersen & Toft 2003; Mayntz et al. 2005; Mayntz & Toft 2006). However, using a split-clutch design, my results demonstrate significant differences among clutches in the effects of prey nutrient content on both mass and body size of two spiders with different life history strategies. Some clutches showed large responses to prey nutrient content, while others showed no significant difference between individuals on the low and high diets. Depending upon the clutch, individuals on the high nutrient diet were 12–106% larger than individuals on the low nutrient diet (Fig. 2). Interestingly, there were no signifieant differences between clutches in the mass or body size of spiders on the low nutrient diet. The differences among clutches depended upon how large spiders grew when fed the high nutrient diet. The differences among clutches in the effects of diet were unexpected, given that individuals of both species were collected from relatively small geographic areas (*T. helluo* from one 13 ha field and *P. phalangioides* from one isolated population in a building). The large variation among clutches and qualitative differences in whether or not clutches significantly responded to diet treatment in this study highlight the importance of including this factor in studies of the effects of diet on survival and growth.

Clutch by diet interactions could have been a consequence of maternal or genetic effects (Mousseau and Dingle 1991). For maternal effects, adult females with different nutritional histories could influence the growth or survival of their offspring, depending upon the type or amount of nutrients provisioned to eggs (Mousseau & Dingle 1991; Bernardo 1996). The effect of maternal environment or condition on offspring performance has been demonstrated in a wide range of animals (Mousseau & Dingle 1991). For example, in spiders, sexual cannibalism of a small male by a female can affect the size, growth and survival of the subsequent offspring (Rabaneda-Bueno et al. 2008; Welke & Schneider 2012). More recent work has also highlighted the potential for epigenetic modification of gene expression in offspring, depending upon the conditions experienced by the mother (Bossdorf et al. 2008). However, the possibility of strong maternal effects in the current study was lessened by the fact that females used in this study were fed similarly before egg production.

Another explanation for these effects is that they were due to genetic variation in response to diet nutrient content. For example, in *Daphnia* spp. there is evidence that longer intergenic spacer (IGS) regions in the rDNA tandem repeat unit can result in higher rates of rRNA transcription and higher organismal growth rate (Elser et al. 2000; Weider et al. 2004). For example, Weider et al. (2005) demonstrated the existence of two clones of *Daphnia pulex* that differed in their response to food quality and the length of the IGS regions in their rDNA. When held under constant conditions in the laboratory, the long IGS region clone competitively excluded the short IGS region clone under conditions of high food quality, and the short IGS region clone excluded the long IGS region clone under conditions of low food quality (Weider et al. 2005). In spiders, Uhl et al. (2004) used a full-sibling design to demonstrate significant interactions between genotype and food quantity on the growth of juvenile *P. phalangiodes*. Similar full-sibling designs could be used to test for interactions between genotype and prey nutrient content using a wide range of nutrient manipulations.

Regardless of the mechanisms responsible for these effects, these results demonstrate significant differences among clutches in the effects of diet on spider growth, even among spiders collected from a limited geographic area. Future studies of spider nutrition should sample a wide range of individuals and explicitly incorporate clutch effects into the statistical design to 1) avoid potential spurious effects (i.e., due to a predominance of a particular genotype that is especially responsive or unresponsive), 2) capture the full range of variation in treatment effects, and 3) ensure that the results of studies are more generalizable.

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Functional aspects of genital differences in *Leucauge argyra* and *L. mariana* (Araneae: Tetragnathidae)

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Abstract. Morphological studies have documented the tendency for male genitalia to diverge rapidly compared to other body parts in many animal groups, including spiders. But documentation of how differences in genital structures of closely related species correlate with differences in the behavior of their genitalia during copulation is rare. This study describes how the genitalia of the spider *Leucauge argyra* (Walckenaer 1841), a species in which both male and female have unusual derived structures, are used during copulation and compares their sexual behavior with previous descriptions of genital behavior in the congener *L. mariana* (Taczanowski 1881) and the genital morphology of other *Leucauge* species. Males of *L. argyra* have two prominent derived genital structures, both of which interact directly with the female; one of them apparently locks against a modified female structure, while the other is inserted into the female atrium. On the other hand, the most prominent derived female structure does not lock against or receive any male structure and may serve to sense movements of the male palp, perhaps to trigger deposition of a strong copulatory plug by the female. The female atrium is unusual in that it receives insertions of both the male's conductor and his cymbial hook. Both derived male structures of *L. argyra* may have evolved to stabilize the male's genitalia during intromission, perhaps in response to violent and dangerous female resistance or to perforate the strong plug that is probably produced or at least moved into place by the female. The rotating and projecting movements executed by male genitalia in *L. argyra*, which as in other spiders are presumably produced by the hydraulic unfolding of complex membranes in the palp, are quite different from the movements of the male genitalia of *L. mariana*. We speculate that in spiders in general, changes in palpal sclerites are often accompanied by changes in the movements of the sclerites, and thus by changes in the unstudied internal membranes of the palp.

Keywords: Copulatory plugs, genital movements, genital evolution

Animal genitalia, especially those of males, frequently show especially rapid divergent evolution compared with other body parts, and they often present relatively complex morphologies (Tuxen 1970; Eberhard 1985; Leonard & Córdoba 2010). Despite the abundant documentation of these two morphological patterns in the taxonomic literature of many groups of animals, much less is known about how the rapidly diverging structures of males and females behave during copulation and the evolutionary origins of the diversity. Web-building spiders are a rewarding group in which to study genital behavior, because they can often be induced to copulate with their ventral sides upward under a dissecting microscope, where their genitalia and their movements are easily visible (Eberhard 2004). In addition, most male structures remain outside the female genitalia during mating, where their movements and the coupling mechanisms can be observed.

Although the male and female genitalia of spiders in the tetragnathid genus *Leucauge* White 1841 are not particularly complex compared with those of many other araneoids, they have nevertheless diverged relatively rapidly compared to other structures, as testified by the fact that they are often diagnostic for distinguishing related species (Hormiga et al. 1995; Levi 1980, 2007, 2008; Tso & Tanikawa 2000; Yoshida 2009; Álvarez-Padilla & Hormiga 2011). Previous studies (Eberhard & Huber 1998; Méndez 2004; Aisenberg 2009; Aisenberg & Eberhard 2009) described the movements and physical interactions between the male and female genitalia of *L. mariana* (Taczanowski 1881) during copulation, and how copulatory plugs are deposited and removed (for a general review of copulatory plugs, see Uhl 2010). The present study describes similar details in a second species, *L. argyra* (Walckenaer 1841), which differs strikingly in both male and female genital morphology (Levi 2008; Álvarez-Padilla & Hormiga 2011). We will show that, contrary to expectations, some apparently derived features of the male and female genitalia in *L. argyra* do not interact directly with each other during copulation, raising interesting questions regarding their functions and how they evolved.

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METHODS

Field samples.—We collected *L. argyra* from September through November 2009 in plantations of African oil palm (*Elaeis guineensis*) in Parrita, Puntarenas Province, Costa Rica (09°30′N, 84°10′W; elevation 10 m), and observed them at the Escuela de Biología, Universidad de Costa Rica, San José Province, Costa Rica (9°54′ N, 84°03′ W; elevation 1200 m). We observed and photographed each adult female under a Wild model M3Z dissecting microscope (Wild, New York, USA) to check for the presence of copulatory plugs on the epigynum. Cephalothorax lengths were measured on specimens in ethyl alcohol. We photographed the genitalia of *L. argyra*, as well as those of *L. mariana* (collected near San Antonio de Escazú, Costa Rica), and *L. venusta* (Walckenaer 1841) (collected near Baton Rouge, Louisiana, USA.) with a Hitachi Model S-570 scanning electron microscope (SEM).

Copulatory plugs and spermathecae.—We removed thirty six copulatory plugs from *L. argyra* epigyna using a sharp thin needle and mounted each one on a microscope slide. We stained them with acetocarmine, which stains DNA red but does not stain the plug matrix, to check for sperm. We photographed the preparations under a Leica DME light microscope.

To check sperm stores in adult females captured in the wild, we dissected the epigyna of 31 adult females with a copulatory plug and 32 females without a copulatory plug. Dissections were performed one to five days after the spiders were collected. We removed both spermathecae of each female and mounted them on a microscope slide in a drop of saline solution. Then we placed a cover slip on top and pressed, causing the sperm to emerge from the thin-walled spermathecal chamber I (Quesada & Triana unpubl.). Sperm were active in the saline solution, facilitating detection of both active and inactive cells.

Genitalia and sexual coupling.—We flash-froze two mating pairs of *L. argyra* with ethyl chloride during palpal insertion. The male genitalia did not remain coupled to the female and were preserved in ethyl alcohol; the basal hematodocha collapsed somewhat, but we were nevertheless able to determine the approximate positions of palpal sclerites during copulation. We obtained additional details by clearing two palps in 10% KOH, by dissecting two others, and by making plasticine models of genital structures. We also made video recordings of 12 matings using a SONY DCR TRV50 digital video camera (SONY, San Diego, California, USA) equipped with +4 close-up lenses, and of two additional pairs under a dissecting microscope in which the camera recorded through the ocular and was focused on the epigynum in posterior and slightly ventral view (the view varied somewhat when the animals moved slightly). Specimens were prepared for viewing with SEM using standard procedures.

Data are presented as median ± quartile when we used non-parametric tests and mean ± SD when we used parametric tests. The statistical analyses were performed with Past Palaeontological Statistics, version 1.18 (Hammer et al. 2003), NCSS 2001 (Copyright 2000 Jerry Hintze). Descriptions of genital behavior use the female's body as reference; thus, a "medial" movement of the male palp refers to its orientation with respect to the female's rather than with respect to the male's body. Voucher specimens were deposited in the Museo de Zoología of the Escuela de Biología in the Universidad de Costa Rica.

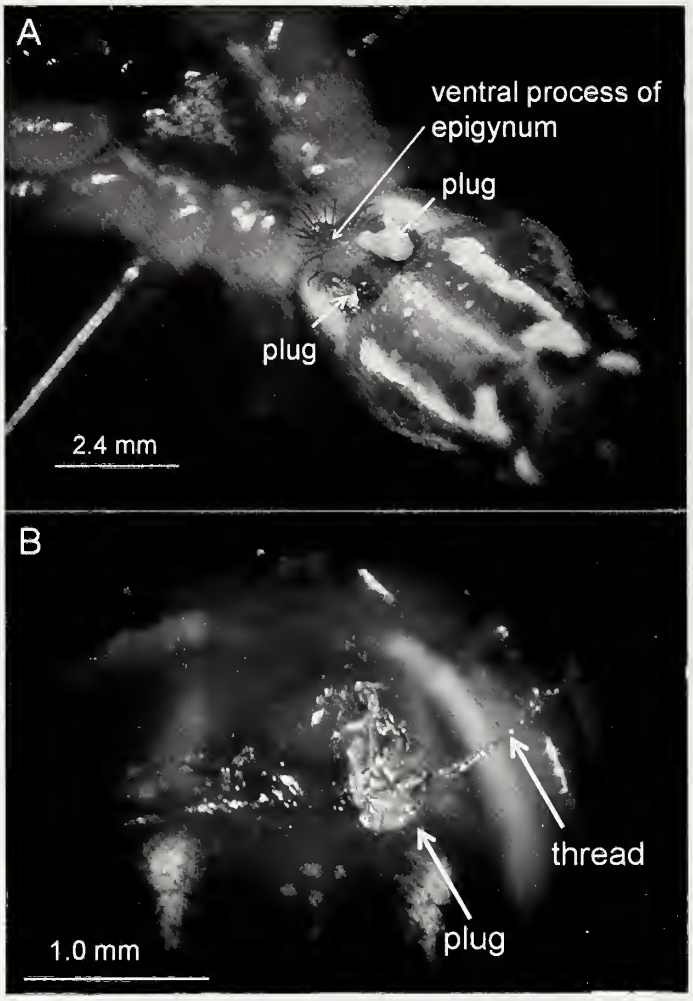

Figure 1.—A) Posterior-ventral view of the ventral epigynal process and its long setae of a mated female, with copulatory plugs of different sizes (arrows) covering the two atria; B) a copulatory plug with a long thread on the left opening of an insemination duct. Such threads are formed when the cymbial hook of the male's palp becomes stuck to the plug material and the male pulls his palp away (ventral-posterior view).

RESULTS

Field samples.—We captured a total of 210 adult females and 98 males of *L. argyra*. Five females laid an egg sac during transportation from the field to the laboratory. Two of these five females had a copulatory plug in the epigynum. Of the other 205 adult females, 113 (55.1%) had copulatory plugs (Fig. 1A). Twenty-four of these plugs (21%) were drawn into one or more thin threads (Fig. 1B): these are indicative of male pedipalp adhesion to the newly formed plug, coinciding with a previous report on captive specimens (Aisenberg & Barrantes 2011).

Copulatory plugs and spermathecae.—Of the 36 copulatory plugs we stained, 12 lacked sperm and were formed exclusively by a matrix of unknown composition, eight consisted mainly of matrix (ca. 95%) with very low numbers of decapsulated sperm, and 16 consisted mainly of a matrix (ca. 95%) that contained low numbers of both encapsulated and decapsulated sperm (Fig. 2). Of the 63 females collected in the field and checked for sperm in their spermathecae, all of the 31 females with copulatory plugs had sperm, and 75% of the 32 females without a copulatory plug had sperm. In all cases, the sperm were abundant (probably hundreds or thousands).

Genital morphology.—One of the most pronounced differences between the male genitalia of *L. argyra* and those of other *Leucauge* species is the large, dorsally directed hook on the antero-dorsal margin of the cymbium (Levi 2008; Figs. 3, 4A) (hereafter the "cymbial hook"; this is the "huge macroseta" mentioned by Álvarez-Padilla & Hormiga 2011 for this species). The hook is apparently a modified seta, as it has an apparent socket at its base (Fig. 4B), and it also broke off easily as a unit in specimens preserved in ethyl alcohol. Its distal exterior surface is covered with many small, distally directed teeth (Figs. 4C, 5A). No aperture was visible near the tip of the hook (Figs. 4A, 5C), nor in the specimen figured by Álvarez-Padilla & Hormiga (2011). There is also a smaller, tooth-like process on the margin of the cymbium (hereafter the "cymbial tooth") with a small indentation near its base (Fig. 4C; this is the "cymbial dorso-basal process" figured for *L. argyra* by Álvarez-Padilla & Hormiga 2011). Because we

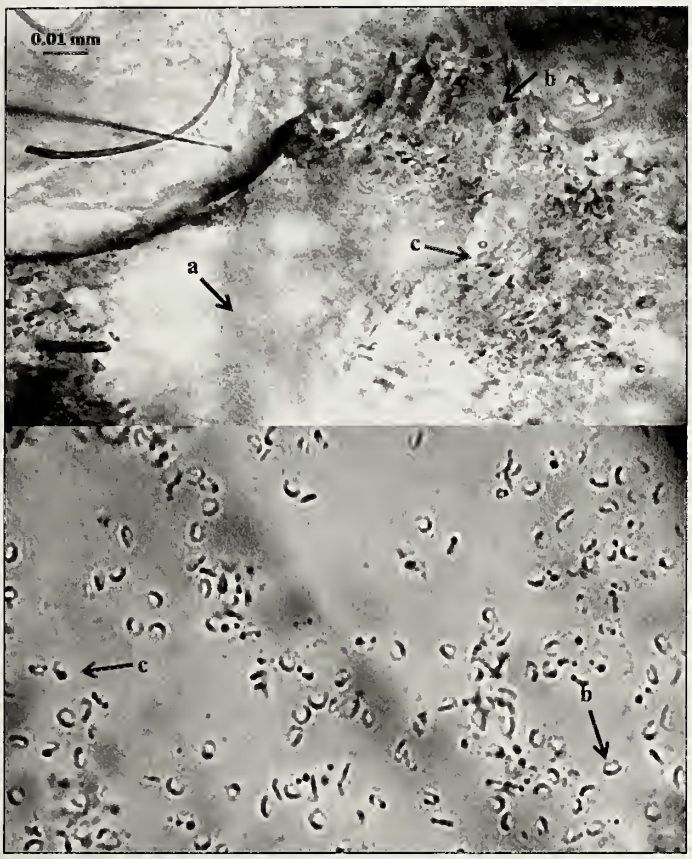

Figure 2.—Above. Copulatory plug stained with acetocarmine, indicating a) the matrix material, b) encapsulated sperm and c) decapsulated sperm; below: sperm that has emerged in saline solution from the spermathecal chamber I of a field-collected female, showing encaspulated sperm and decapsulated sperm.

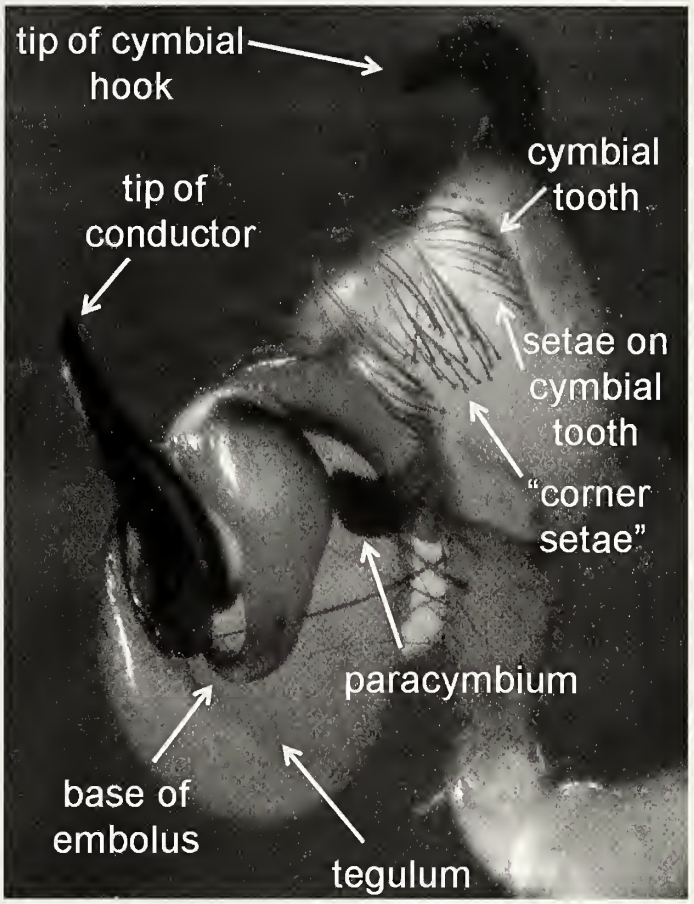

Figure 3.—A photograph of an expanded pedipalp as it would be seen in latero-anterior view during copulation. The relative positions of the conductor and the cymbial hook are somewhat more natural than those in Fig. 4A, as the specimen had not been dehydrated.

are uncertain of homologies, we use only descriptive names here. The outer surface of the tooth bears approximately six long setae in an approximate row (hereafter "tooth setae") (Fig. 4). There is another area with a concentration of long setae at the basal corner of the approximately triangular cymbium (hereafter "corner setae") (Fig. 3).

The most distinctive trait of the epyginum of *L. argyra* is the large conical ventral process of the central posterior portion of the epigynum (hereafter the "ventral process"). It is provided with dense, long setae on its anterior surface, especially near its tip and around its base, but is naked on its posterior surface (Figs. 1A, 6). A second, much less conspicuous feature is a small ridge along the lateral and antero-lateral margin of the epigynum (Fig. 6B) (hereafter the "epigynal ridge"). The atrium is located on the base of the ventral process, just posterior to the anterior (setose) surface (Fig. 6). The opening of the insemination duct is on the medial side of the atrium, and the duct is directed more or less medially.

Sexual coupling and genitalia.—As in *L. mariana* (and other tetragnathids – see Crome 1954; Huber & Singlet 1997; Álvarez-Padilla & Hormiga 2011), the female faced the male and grasped his sexually dimorphic chelicerae with hers just prior to genitalic coupling, following an exchange of courtship vibrations (Aisenberg 2009; Aisenberg & Eberhard 2009). Cheliceral coupling occurred after the female spread her chelicerae and extended her fangs; the male then inserted the distal portion of the basal segment of each of his chelicerae into the space between the female's open fang and her basal cheliceral segment. The female then closed her fangs to clamp the male, and he immediately extended one pedipalp anteriorly toward her genitalia and attempted palpal insertion.

Genital coupling consisted of two stages – insertion of the cymbial hook into the ipsilateral atrium, followed by insertion of the conductor and embolus into the other, contralateral atrium. At the beginning of the first stage, the male extended his palp toward the female's epigynum with its distal portion rotated medially about 90° so that his cymbial hook was directed toward her epigynum (Fig. 7). The male moved the entire bulb laterally (e.g., left and right) back and forth across the epigynum from one to three times in this rotated position, apparently searching to contact the epigynum with the cymbial hook. The basal haematodocha was not perceptibly inflated at this stage, and the palp moved as a unit. On one occasion a favorable viewing angle allowed us to see that the cymbial hook snagged briefly on the ventral process of the epigynum, with its tip on the posterior surface of the process. On the next pass, the bulb contacted the epigynum, and the cymbial hook contacted the atrium of the epigynum; the male immediately re-positioned the hook slightly as it penetrated deeper into the ipsi-lateral atrium. As soon as the cymbial hook was inserted in the atrium, the basal haematodocha

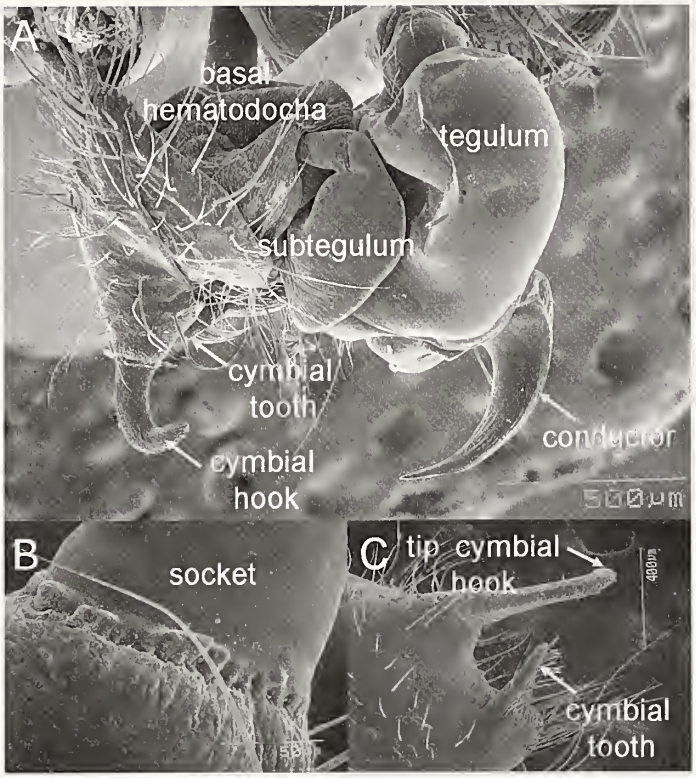

Figure 4.—SEM photos of male palps. A) A partially expanded palp seen as it would be in a posterior view of a copulating pair, illustrating the tong-like positions of the conductor and the cymbial hook. The basal hematodocha is partially collapsed, and the cymbial hook has twisted somewhat toward the viewer. B) Close up view of the base of the cymbial hook. C) Close up view of cymbial tooth showing its setae and also the teeth on the tip of the cymbial hook.

inflated rapidly. This expansion produced a ventromedial rotation of the tegulum, the conductor and the embolus away from the cymbium. This brought the medially directed, curved conductor into the contralateral atrium of the epigynum (Fig. 7). Thus the tips of the cymbial hook and the conductor pressed in approximately opposite directions, each into an atrium (Figs. 4A, 7), with each tip directed medially and slightly posteriorly. Although the tips of the conductor and the cymbial hook were not perfectly opposed, the overall mechanical effect was to pinch the female's epigynum as if with a pair of tongs. The cymbial tooth and the small indentation at its base were not visible in the posterior views in our video recordings, so it was not possible to observe their mesh with the female directly. Nevertheless, the recordings, SEM photographs that provided approximate scales, and manipulation of plasticine models allowed some deductions. The tooth and the indentation did not mesh with sides of the ventral process (confirmed by direct observation in the videos). They also did not mesh with the rim of the atrium, because the indentation was too far from the cymbial hook and the rim was too close (in addition, the orientation of the tooth was inappropriate – it was nearly parallel to the rim, rather than perpendicular to it). One further possibility was that the tooth and the indentation hooked onto the epigynal ridge when the cymbial hook was inserted into the atrium (Fig. 7). This area was never directly visible in recordings that provided sufficient magnification (two made through the dissecting microscope); but manipulation of the models showed that if the cymbial hook was inserted deeply and the insemination duct was directed slightly anteriorly, the tooth and its indentation would have been positioned exactly over the epigynal ridge. One further detail favoring this hypothesis was that in this position the setae on the cymbial tooth would have been directed toward the female epigynum; they would thus have been in position to function, allowing the male to sense the presence of the ridge and thus orient his palp.

Rhythmic inflation of the haematodochae and palpal sclerite movements.—Once the tip of the conductor was inserted into the atrium, the palp executed a stylized sequence of movements (0.97 ± 0.11 s, n = 11) that rhythmically withdrew and then reinserted the cymbial hook into the atrium. The sequence began with a partial collapse of the basal haematodocha. As the haematodocha gradually collapsed, the edge of the cymbium was displaced medially toward the tegulum, and the cymbial hook was lifted out of the epigynal atrium (Fig. 7B). This movement brought the medial edge of the cymbium near or sometimes slightly past the middle of the female's ventral process (Fig. 7B). At the same time, the tegulum moved slightly toward the cymbium (the movement of the tegulum was much smaller than that of the cymbium). Toward the end of the collapse, the embolus base moved away from the atrium over about 0.39 ± 0.11 s (n = 11) (visible only with certain viewing angles and indicated by the arrows in Fig. 7), indicating that the tip of the embolus was retracted gradually. The tip of the conductor remained inside the atrium.

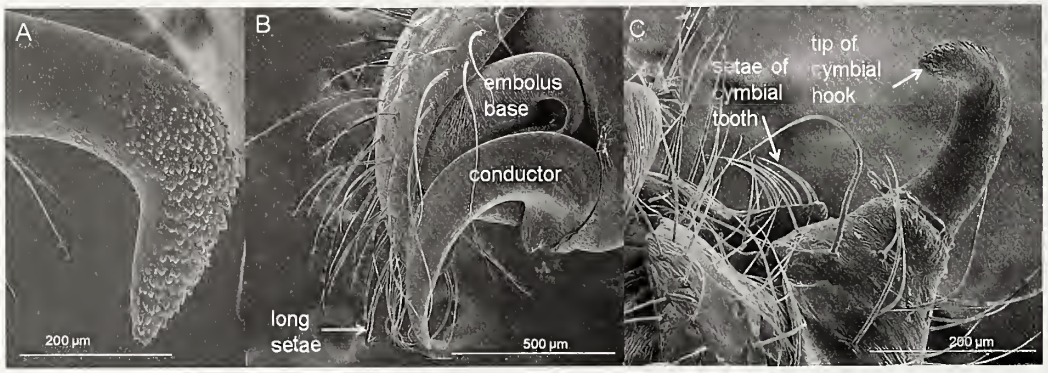

Figure 5.—The pedipalp of a male *L. argyra*. A) Closeup of the teeth on the tip of the cymbial hook; B) long setae (arrow) close to the tip of the conductor in an unexpanded palp; C) long setae on the cymbial tooth.

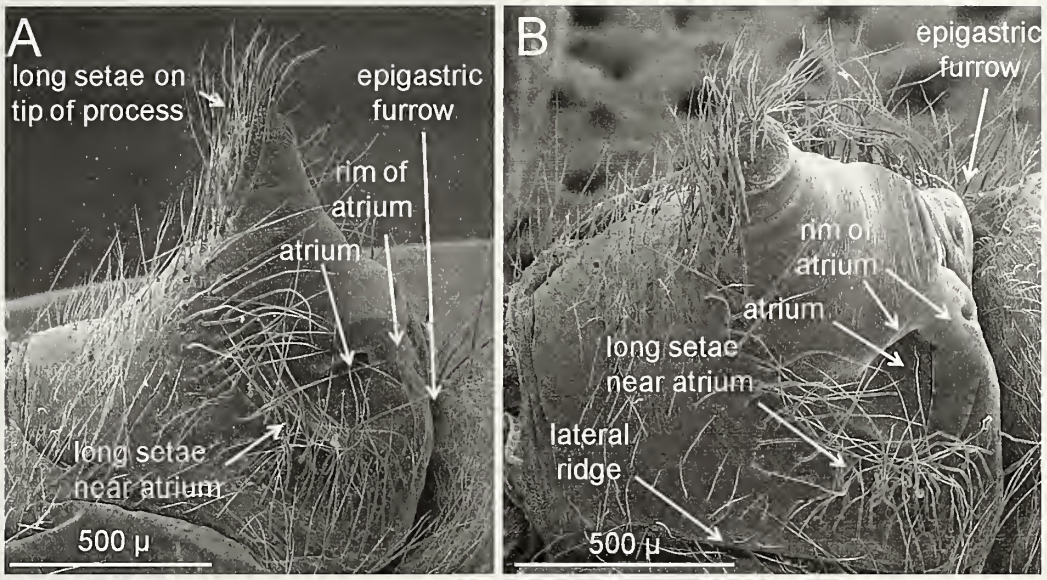

Figure 6.—Epigynum of *L. argyra*. A) in lateral view; and B) in latero-ventral view, showing the wide atrium and long setae anterior and lateral to the atrium.

The second portion of the sequence began with reinflation of the basal haematodocha. The inflation was more abrupt (lasting a mean of 0.28 ± 0.05 s, $n = 11$) than the collapse (lasting a mean of 0.64 ± 0.10 s, n = 11). Inflation reversed the movements just described. Approximately 0.20 s (± 0.02, $n = 11$) after expansion began, the cymbial hook moved rapidly back into the epigynal atrium (and small cymbial tooth and its accompanying indentation may have hooked on the epigynal ridge). In some cases, as much as the distal half of the cymbial hook disappeared into the atrium. At the same time the tegulum moved slightly away from the cymbium. After the haematodoeha had begun to reinflate, the embolus began to move into the insemination duct 0.034 ± 0.009 s ($n = 11$), basically at the same time that the hook began to descend to the atrium; it took 0.15 ± 0.02 s ($n = 11$) for the base of the embolus to disappear out of sight (Fig. 7C).

It is very likely that the movements of the cymbium bring it or its setae (and perhaps also the tegulum) into contact with at least one set of the especially long setae that are associated with the ventral process of the epigynum – those at its base, near the atrium. The in-and-out movements of the cymbial hook must inevitably deflect setae at the edge of the atrium (Fig. 6). The area of the cymbium near the base of the cymbial hook likely defleeted setae on the anterior surface of the ventral process. The especially dense group of long, ventrally directed setae near the tip of the ventral process (Fig. 6A) was not clearly contacted by the male palp, however, though it is possible that they were touched by setae on the cymbium.

Comparisons of sexual behavior and copulatory plugs between *L. argyra* and *L. mariana*.—Data on several aspects of the sexual behavior of *L. argyra* can be compared with those of *L. mariana* (data on *L. mariana* are taken from Aisenberg 2009; Aisenberg & Eberhard 2009). The males of *L. argyra* are relatively larger (relative to eonspecific females) than are those of *L. mariana*. Comparing the degree of sexual dimorphism in cephalothorax length (male/female), the respective medians and quartiles were 0.98 ± 0.16 (0.59–1.25), $n = 26$) for *L. argyra*; and 0.87 ± 0.13 (0.61–1.17), $n = 43$ for *L. mariana* (Student t Test: $t = 3.09$, $df = 67$, $P = 0.003$). The relatively larger size of *L. argyra* males may be related to the greater danger that females represent for males in this species, in which the frequency of sexual cannibalism was greater (11.1% of 45 pairs in *L. argyra*, 0% of 62 in *L. mariana* ($\chi^2 = 7.23$, $df = 1$, $P = 0.007$). Vigorous struggles associated with copulations, in which the female appeared to try to grasp the male and the male appeared to try to escape, but which did not end in eannibalism, were also more common in *L. argyra* (10 out of 12 copulations with previous virgins and five out of five copulations with mated females); no such struggles occurred in 43 pairs of *L. mariana* with virgin females ($\chi^2 = 43.8$, $df = 1$, $P = 0.0001$), or in 18 pairs of *L. mariana* with non-virgin females ($\chi^2 = 23.0$, $df = 1$, $P = 0.0001$). These data are all from matings in captivity, but we also saw attacks on males by female *L. argyra* in the field.

The overall frequency of intromission attempts that failed ("flubs") was lower in *L. argyra* than in *L. mariana* ($U = 63$, $n_1 = 11$, $n_2 = 43$, $P = 0.0002$). However, in *L. mariana* flubs were less frequent with long than short intromissions, and *L. argyra* performed only long intromissions (the mean number of long, cymbial insertions per mating in 12 copulations was 2.54 ± 1.13, while the number of hematodochal inflations per insertion was 83.3 ± 56.9), so this comparison may not be appropriate. When we took this difference into account by creating an index of the number of flubs/the number of insertions for each mating, and using only long insertions in *L. mariana*, the respective means of the indices were still statistically different (0.52 ± 0.69 in *L. argyra* and 5.4 ± 7.2 in *L. mariana*; $U = 70.5$, $n_1 = 43$, $n_2 = 12$, $P = 0.0004$). In fact, the flubs of *L. argyra* were limited exclusively to the preliminary attempts to first insert the cymbial hook into the atrium; once this engagement occurred, the insertion attempts with the conductor that followed, resulting from hematodochal expansion (i.e., the movements homologous to insertion attempts in *L. mariana*) never failed.

Copulatory plugs are formed during copulation or in the following hours in both *L. argyra* and *L. mariana* (75% of 12

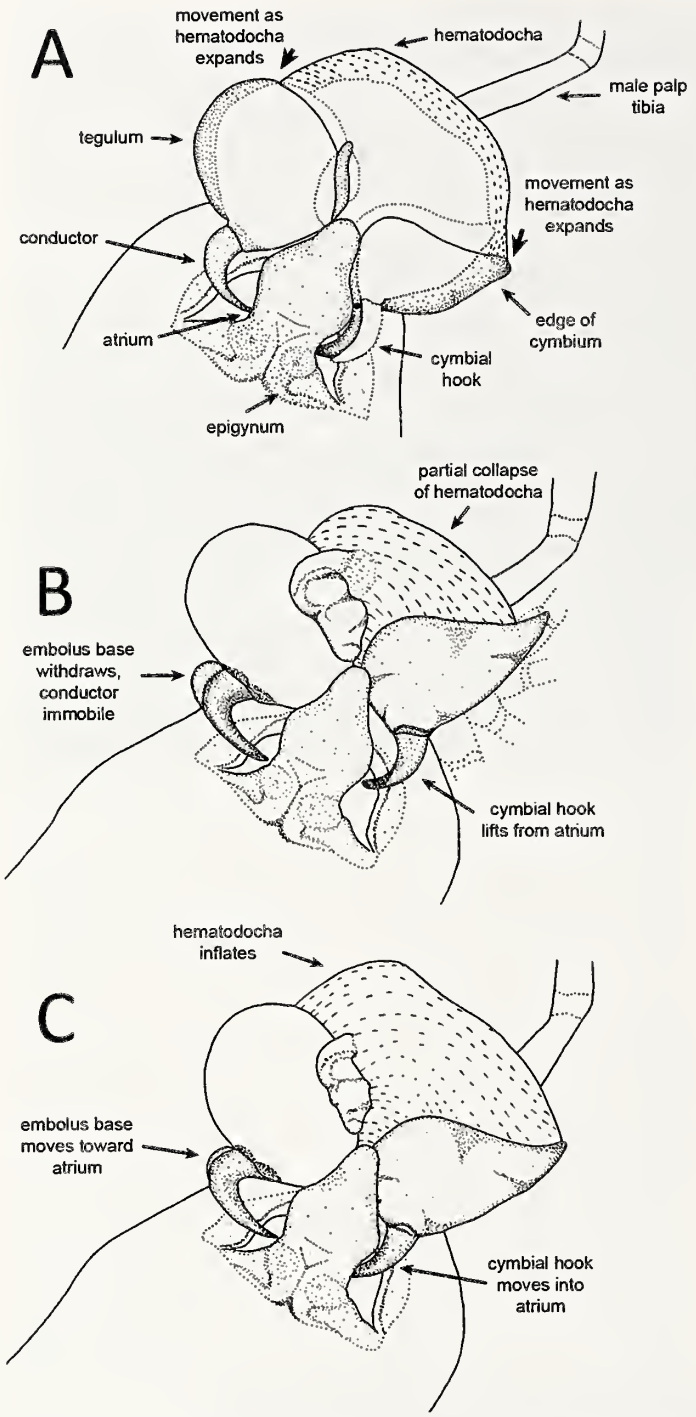

Figure 7.—Partially schematic posterior-ventral view of female and male genitalia during palpal insertion (A) and movements of the sclerites of the left male bulb during collapse and inflation of the haematodochae (B) and (C). The dotted lines in (A) indicate positions when the hematodocha is collapsed.

first copulations for the female in *L. argyra*, 44.2% of 43 in *L. mariana*) ($\chi^2 = 3.56$, $df = 1$, $P = 0.06$). In both species the plug is sometimes but not always effective in preventing intromission by a second male (71% of 7 cases in *L. argyra*, 53% of 19 in *L. mariana* ($\chi^2 = 0.19$, $df = 1$, $P = 0.46$). Testing the consistency of plugs of field-collected females by probing them with a fine needle indicated that those of *L. argyra* are harder and adhere more tenaciously to the epigynum; when female setae were embedded in a plug of an *L. argyra*, the plug could usually only be pulled away from the female by breaking off the setae, which remained embedded in the plug (N. Caballero & A. Aisenberg unpubl.). We did not carefully test whether the consistency of *L. argyra* plugs changed over time; they remained hard once they had solidified, because the plugs in females collected in the field and kept for multiple days afterward were hard.

Comparisons of *L. argyra* genital morphology with that of other *Leucauge* species.—Our observations in the SEM of the male and female genitalia of *L. argyra, L. mariana* and *L. venusta* permit comparisons of many details. In the epigyna of all three speeies (Figs. 6, 8, 9) there is a setose anterior region, and a naked posterior region, where, at least in *L. argyra* and *L. mariana,* the palpal sclerites press against the female. The setose anterior region of *L. mariana* ends abruptly at the shallow wall that marks the anterior edge of the naked region (Fig. 8), while that of *L. venusta* extends posteriorly, forming a hood that partially covers the naked posterior region. This hood has a pair of depressions with a knobby internal surface whose functional significance remains to be determined (Fig. 9). The anterior surface of the large ventral projection of *L. argyra* is setose and is thus apparently an extension of the anterior region, if one can use setae as markers for these regions; the posterior surface of the projection is completely naked (Fig. 6B).

Some of the epigyneal setae of *L. argyra* are relatively longer than those of either *L. mariana* or *L. venusta* (Figs. 6, 8, 9). The longest setae occur near the tip of the epigyneal projection and along the lateral margins near the atria, toward which they project. The setae are denser in the areas where they are longest. The epigynal setae of the other two species are more uniform in distribution and length, although *L. venusta* has small patches of setae that project toward the naked area on the lateral portions of the posterior edge of the hood (Fig. 9A).

The entrances of the insemination ducts of *L. argyra* are relatively exposed compared with those of *L. mariana* and *L. venusta,* which are somewhat protected by epigynal structures – the lateral plates of *L. mariana* (Fig. 8), and the hood of *L. venusta* (Fig. 9). As far as we know, the atrium of *L. argyra* is unique among spiders in receiving insertions by two different male palpal structures, the conductor and the cymbial hook. The lateral epigynal ridge of *L. argyra,* which may engage the male cymbial tooth, is apparently absent in the other two species.

The male genitalia of *L. argyra* differ from those of the other species with respect to both the cymbium and the palpal bulb. There were no signs of either projections or indentations on the cymbia of *L. mariana* or *L. venusta* that might correspond to the cymbial hook or the cymbial tooth of *L. argyra.* The form of the paracymbium is similar in all three species; its position in the expanded palp of *L. argyra* (Fig. 3) suggests that it functions not to contact the female but to push against the embolus base, as occurs in *L. mariana* (Eberhard & Huber 1998).

Comparisons are also possible with recent published (Tso & Tanikawa 2000; Yoshida 2009) and digitally published (Levi 2007) taxonomic drawings of the genitalia of 44 other *Leucauge* species beside the ones we studied (42 species of females, 15 species of males). Less detail is available because

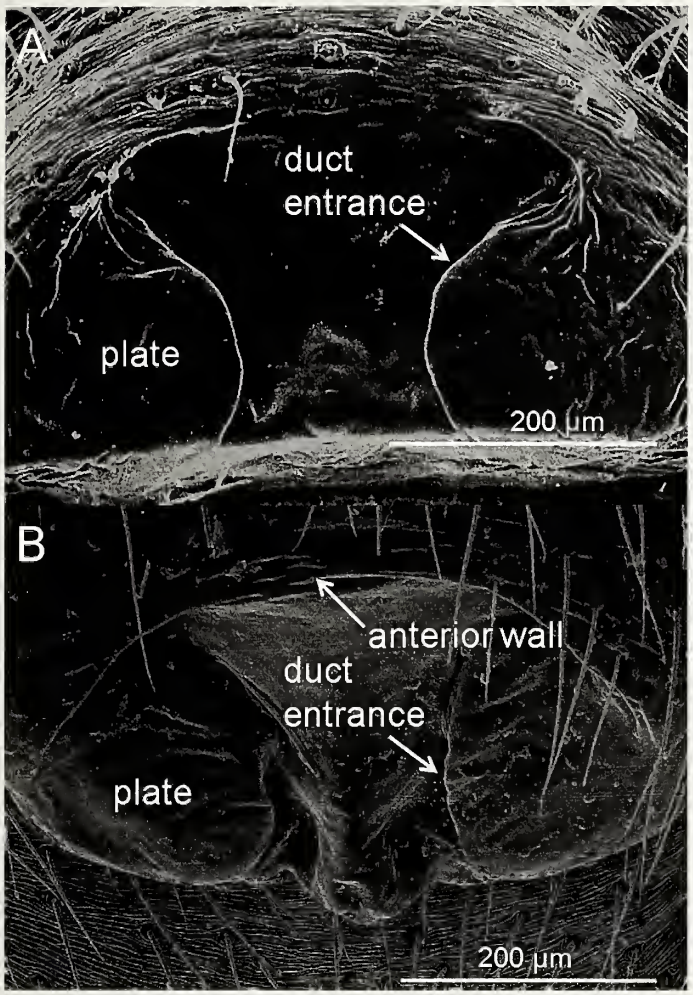

Figure 8.—SEM images of unplugged epigyna of *L. mariana* seen in A) ventro-posterior view, and B) latero-posterior view. The small hood over the anterior wall of the atrium, the lateral plates, and the entrance to the insemination duct are visible. The rear edge of the epigynum in B is tilted more strongly away from the ventral surface of the abdomen than is that in A.

not all structures are visible in all of the drawings, and because the setae are omitted in nearly all cases. In none of the 42 species in which the epigynum was drawn is there any elevation similar to or even suggestive of the ventral process of *L. argyra*, nor do any have such exposed, unprotected atria as those of *L. argyra*. There was also no epigynal ridge in any other species, but this structure is cryptic, so it could have been omitted from drawings. In only one of the 15 species of males, *L. ocellata* (a junior synonym of *Metabus ocellatus* Platnick 2013) is there a cymbial hook. This hook is approximately the same size as that of *L. argyra* and in a similar position on the eymbium, except that it is located less basally (about one-third of the distance to the distal tip). There is no cymbial tooth or indentation visible in *M. ocellatus* or in any other species. The atrium of *M. ocellatus* is relatively unexposed and is more similar to that of *L. mariana* than that of *L. argyra.*

DISCUSSION

Sperm plugs and their origins.—Our observations of *L. argyra* agree in some respeets with the previous conclusion of Aisenberg & Barrantes (2011) that the copulatory plugs of this species are usually if not always produced by the female rather than by the male. The plug material usually lacked sperm completely, had at most only a small fraction of sperm, and sometimes had unencapsulated sperm, all indicative of a female origin for the plug. The contents of the sperm duct of *L. mariana* were densely loaded with sperm, all of which were encapsulated (Méndez 2004). Nevertheless, it is not impossible that male material transferred to the female in *L. argyra* lacks sperm, so this is not conclusive evidence. Much of the wall of the soft chamber I of the spermatheca of *L. argyra* is apparently glandular, and it could be the source of the plug material (Quesada & Triana, unpubl.) (spider spermathecae in general often have associated glands, however, so this is also inconclusive evidence – see Eberhard & Huber 2011).

It is not clear, however, how a female, having just received an ejaculate of spermatozoa that largely fills the lumen of chamber I of her spermatheca, can then move gland products produced by the walls of this chamber to the external surface of her epigynum without the gland products becoming mixed with the sperm that the male has just deposited near the spermathecal entrance. It would seem that there must have been sperm in chamber I when the plug formed on the surface of her epigynum; the sperm are not eliminated or moved elsewhere soon after copulation, because we frequently observed sperm in the spermathecal chamber I in females several days after they were collected in the field and isolated from males.

This puzzle could be explained if the plug material were derived from the male rather than the female. If it were transferred after the sperm were transferred and did not mix with the sperm inside the female, it would be possible to explain the observation that some plugs appear only hours after the end of copulation (Aisenberg & Barrantes 2011). One problem with this hypothesis is that the sizes of some sperm plugs seemed too large to be housed, along with the sperm volume that is stored in chamber I, in the male's palp. Perhaps the plug material includes instead a combination of male and female products as in *L. mariana* and is formed when some female component of the plug material crosses the walls of the insemination duct to mix with the male product, and then the combination emerges onto the surface of her epigynum. The long delays between copulation and plug formation (often many hours), and the direct observations of plug material welling up from inside the female and then hardening (Aisenberg & Barrantes 2011) make it difficult to believe that there is not some sort of active female participation in the process.

Evolution of new genital structures.—The phylogeny of species in *Leucauge* is not known; to our knowledge, this large genus has never even been revised. Many details of evolutionary transitions thus cannot yet be determined. Nevertheless, some prominent structures in *L. argyra* are apparently unique to this species, so it is possible to use the behavioral and experimental data from this and other studies to make some preliminary deductions.

Although the ventral epigynal process of *L. argyra* is large and prominent, there is no sign of any similar structure in any of the other species of *Leucauge* that we checked. This process appears to represent a ventral projection of the posterior area

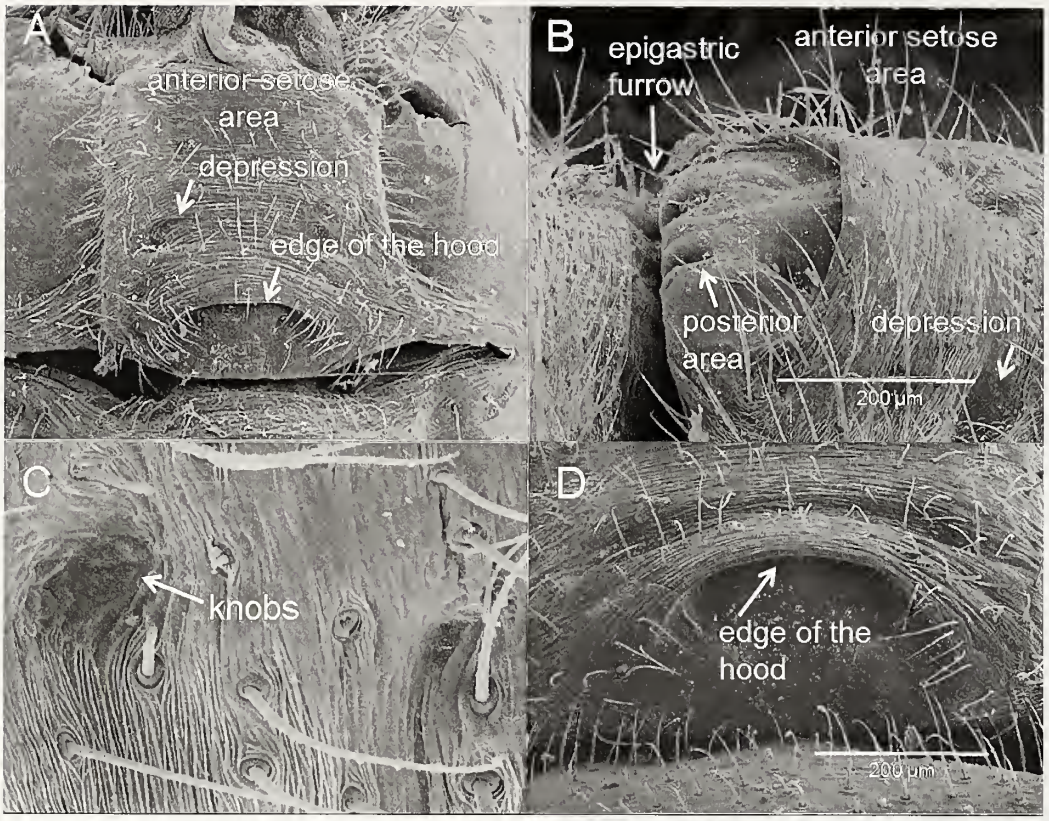

Figure 9.—SEM images of the epigynum of a *L. venusta* seen in A) ventral view, B) posterior-lateral view, C) close up view of depression in the anterior portion and D) posterior view. The epigynum in B) is tilted farther away from the epigastric furrow than is the epigynum in D).

of the anterior, setose region of the epigynum and of the anterior area of the posterior, naked region (see Figs. 6, 8, 9). The epigyna of other leucaugine tetragnathids such as *Chrysoneta alajuela* Levi 1986 and *Azilia affinis* O.P.-Cambridge 1893 also have a setose anterior region and a naked posterior region, but lack ventral processes (Álvarez-Padilla & Hormiga 2011).

Functionally, the ventral process of the epigynum and its associated setae apparently serve sensory functions. The process does not mesh mechanically with any male structure during copulation. It has no internal structures such as cuticular projections, muscles or glands of obvious functional significance (Álvarez-Padilla & Hormiga 2011; Quesada & Triana unpubl.). It does not represent an obstacle to the male, as may be the case with a similarly protruding epigynal process of the pholcid *Mesabolivar* sp. —see Huber et al. 2005, because the atria of *L. argyra* are on the sides of the process rather than hidden behind it. Instead, the relatively abundant and elongate setae on the ventral process are contacted by the male's palp in one and possibly two contexts. The most certain contact is between the espeeially long and dense setae around the base of the ventral process and the atrium (Fig. 6), and the male's cymbial hook and conductor; they lie in the area through which the cymbial hook passes when it is being inserted and withdrawn from the atrium during copulation. The rhythmic in-and-out movements of the cymbial hook must deflect these setae repeatedly.

One other, less dramatic areas of contact may occur between the setae on the anterior surface of the ventral process, especially near its tip, and the surface of the cymbium or its setae; a second possible area of contact is between the setae nearer the base of the ventral process and the corner setae of the cymbium. Our posterior angle of observation prevented us from distinguishing whether the palp did or did not deflect setae on the anterior surface of the epigynal process. The cymbium moves past the setae on the anterior portion of the female epigynal process as the conductor is moved into position for the first insertion and during the rhythmic in-and-out movements of the cymbial hook. This interpretation is not in accord, however, with the orientation of the setae on the distal anterior surface of the process, especially near its tip. Here they projeet ventrally (distally) rather than anteriorly, as might be expected if they were designed to contact the male's cymbium. These interpretations also leave unresolved the significance of the length of the ventral process. At no point did we see a clear contact between any male structure and the distal portion of the ventral process. We were limited, however, by our angle of viewing, and perhaps also by having close-up recordings of only two copulations.

Experimental immobilization of the epigynal setae on or near the base of the ventral process (which presumably largely prevented their being stimulated by the male) inhibited female production of strong copulatory plugs (N. Caballero & A. Aisenberg, unpubl.). Thus, it may be that by stimulating these setae, the male increases his chances of paternity by affecting cryptic female choice. The morphology of females of *L. venusta* hints that they may also be sensitive to male genital movements, as the epigynum has setae concentrated at the lateral corners of the hood (Fig. 9a). Perhaps elaboration of

the ventral process of *L. argyra* occurred under selection to increase the female's ability to sense male palps and their movements. Further data will be needed to test these ideas.

Cymbial hook and female atrium.—The large cymbial hook of *L. argyra* is apparently unusual in the genus *Leucauge*; this structure is so prominent that it is unlikely to have been overlooked in taxonomic drawings. The hook was inserted into the atrium on the side of the epigynum opposite the atrium into which the conductor and embolus were inserted. The numerous small teeth near the hook's tip (Fig. 5a) presumably serve to increase the friction between the hook's tip and the smooth wall of the atrium or the insemination duct. The mechanical consequence of inserting and anchoring the cymbial hook in one atrium while the conductor and embolus are inserted into the other is that the palpal bulb seizes the female's epigynum as with a pair of tongs. The tong-like grip may anchor the conductor more solidly in the atrium and the insemination duct, perhaps facilitating or stabilizing insertion of the embolus and sperm deposition in the insemination duct.

Insertion of the hook into the atrium also results in the male obtaining a mechanical reference point and thus improving his ability to insert his conductor into the other atrium. This would explain how *L. argyra* avoids the frequent "flubs" made by *L. mariana*. These interpretations do not explain, however, all of the male's behavior, as they do not account for the subsequent rhythmic movements of his cymbial hook into and out of the atrium. In effect, the male rhythmically releases his grip on the female's epigynum and then grasps it again. Two functions for these rhythmic movements occur to us. They may serve to perforate the plug material when the hook grasps an atrium that has a rigid copulatory plug, much as an ice pick is used to break a chunk of ice. An alternative, non-exclusive hypothesis is that the movements serve to stimulate the female. The plug removal hypothesis is in accord with our observation that the conductor, which is the only other male genital structure that is positioned appropriately to perforate plugs in *L. argyra*, is relatively weak and flexible, and seems physically incapable of perforating the hard plug material. On the other hand, it does not explain the long female setae positioned to sense movements of the eymbium (above), nor the distally directed teeth on the tip of the cymbial hook. It would seem that basally directed teeth would be more effective in removing plug material, as in the basally directed spines on the odonate penis that remove sperm (Waage 1983). Perhaps these structures and their movements have both mechanical and stimulatory functions.

The physical coupling of the cymbium with the epigynum prior to insertion of the conductor contrasts strongly with the mechanics of *L. mariana* copulation (Eberhard & Huber 1998). The cymbium of *L. mariana* is not coupled mechanically in any way to the female when the male attempts to insert his conductor and embolus into the epigynum. The rounded "external", setose surface of the male's cymbium is simply placed on the apparently featureless, also sparsely setose surface of the female's abdomen; inflation of the basal hematodocha then causes the conductor and embolus to twist away from the cymbium and the abdominal surface and to roll so that the conductor is driven toward the epigynum; there is no other preliminary contact (Eberhard & Huber 1998). This movement is apparently homologous with the second stage of insertion in *L. argyra* (following insertion of the cymbial hook).

A second clear contrast with *L. mariana* was that female *L. argyra* often struggled violently during copulation, and occasionally killed and cannibalized the male (Aisenberg & Barrantes 2011). The more secure mechanical coupling of the palp to the epigynum in *L. argyra* could have evolved to overcome female resistance, or female resistance could have evolved to test the stability of the male's coupling. But, as just mentioned, the subsequent rhythmic in-and-out movements of the cymbial hook do not make sense as attempts to physically overcome female resistance, so there is more than a physical male-female struggle occurring in *L. argyra*. It is also clear that the added mechanical stability provided by the cymbial hook in *L. argyra* does not come without a cost. In some copulations the male's cymbial hook becomes trapped in the sticky plug material produced by the female, and she kills him (Aisenberg & Barrantes 2011).

The female structure with which the cymbial hook interacts is the atrium. Its wide, flaring form, at least in general aspect, shows no modification that is complementary to the hook's form. The atrium of *L. argyra* is large and much more exposed, however, than the atria of any of the other *Leucauge* species for which we have information, making insertion of a cymbial process mechanically easier in *L. argyra* than it would be in the other species. This possible coevolutionary change in the female genitalia of *L. argyra* is appropriate to favor the corresponding male genitalic structure (the cymbial hook), rather than to defend against its use to anchor the palp to the epigynum. Such apparent "selective cooperation" by the female is typical of genital coevolution in many other groups (Eberhard 2004, 2010). It is compatible with females exercising cryptic choice (Eberhard 1996) by favoring males that have hooks, but is not in accord with the sexually antagonistic coevolution hypothesis for genital evolution (Arnqvist & Rowe 2005).

The atria of the epigynum of *M. ocellatus*, the only other related species in our survey with a cymbial hook, are very different. They are hidden from any direct insertion of the cymbial hook, suggesting that the hook in this species probably has a different, unknown function.

The cymbial tooth and its associated indentation.—The small cymbial tooth and the associated indentation in the cymbial margin are also absent in 17 other species of *Leucauge*. These structures are small, however, and could have been overlooked (they are visible, though not emphasized, in Levi's drawing (1980) of *L. argyra*). Functionally, the small tooth and the associated indentation may be associated with insertion of the large cymbial hook into the atrium. The form of the tooth and the indentation seem designed to hook or snag on some protruding structure. More by a process of elimination than by direct observation, we have concluded that the tooth and the indentation may hook the lateral epigynal ridge when the cymbial hook is inserted into the atrium; they may brace the tooth there more securely. Presumably the long setae on the hook serve as sense organs that inform the male regarding whether his tooth is near the lateral ridge of the female. Tentatively we propose that the cymbial hook evolved before the tooth and its accompanying indentation; the tooth

presumably evolved later to improve the mechanical stability of the hook when inserted into the atrium.

The female modification that may match the eymbial tooth and indentation is the ridge on the lateral and anterior margin of the epigynum. There is no similar ridge in *L. mariana* or *L. venusta.* It is not clearly present in any of the other 44 species, but it is an inconspicuous trait, so its absence in taxonomie drawings does not provide certain evidence. The ridge of *L. argyra* seems designed to increase the purchase of the eymbial tooth and thus to increase the firmness of the coupling of the cymbial hook with the atrium. Thus these male and female structures may have eo-evolved in *L. argyra,* but this is uncertain speculation because our evidence for the mechanical mesh with the ridge is only indirect, and we lack information for nearly all other *Leucauge* species. If our hypothesis is correct, this design of the female functions to selectively cooperate with males, aiding those males that have an appropriate tooth and indentation forms rather than excluding them. It is thus compatible with the eryptic female choice hypothesis rather than the sexually antagonistic coevolution hypothesis for genital evolution.

Judging by the distance moved by the embolus base during copulation in *L. argyra*, the tip of the male's embolus passes through the relatively short insemination duct and enters the basal portion of the large, soft-walled receptacle (spermatheca chamber I), as also appears to occur in *L. mariana* (Eberhard & Huber 1998). The sclerotization of the lining of chamber I at and around its entrance in *L. argyra* (Quesada & Triana, unpubl.) supports this interpretation. Presumably it protects against friction with the embolus tip. The mechanism by which the embolus is moved (pushed by the paracymbium on the embolus base) is also similar in the two species. The more internal portions of the internal female genitalia differ dramatically, however, in the two species. In *L. mariana* there are two rather than one additional hard-walled chambers with finger-like inward projections of their walls, and both are substantially larger than the single chamber II of *L. argyra* (Quesada et al. 2011). These female structures are never contacted by the male genitalia, and the significance of these differences is unclear.

Hematodocha behavior.—The durations of the insertions of the conductor of *L. argyra* differed from those of *L. mariana.* Copulation in *L. mariana* includes two types of insertion: long insertions with multiple inflations of the basal hematodocha during each insertion, which tended to occur early in a eopulation, and were associated with transfer of ejaculate to the female spermatheca; and short insertions with only a single hematodochal inflation, often repeated over and over (associated with deposition of sperm plug material on the surface of the epigynum) (Eberhard & Huber 1998). Only long insertions occurred in *L. argyra*, and males did not obviously transfer copulatory plug material.

There were also sharp differences between *L. argyra* and *L. mariana* in both the patterns of inflation of hematodochae and the sclerite movements that they produced. The tegulum of *L. argyra* first turned about 90° without any perceptible inflation of the basal hematodochae, and only then was the hematodocha inflated to insert the conductor into the atrium. In contrast, inflation of the basal hematodocha rotates the bulb about 180° in *L. mariana* without any prior rotation of the tegulum. In *L. argyra,* subsequent collapses and inflations of the basal hematodocha caused small movements of the cymbial hook in and out of the atrium while leaving the conductor in place. Similar rhythmic inflations and collapses in *L. mariana* produced two distinct movements, both different from those of *L. argyra.* During short palpal insertions the conduetor and embolus of *L. mariana* withdrew entirely from the atrium with each collapse. During long insertions they remained inserted, but the tip of a process on the conductor (absent in *L. argyra*) was rotated to contact the anterior wall of the atrium with each inflation. The median hematodocha caused further movement in *L. mariana,* but it was never seen to be inflated in *L. argyra.* Collapsing the median hematococha in *L. argyra* did not produce exactly the inverse sequence of the movements produced by inflation, as appears to occur in *L. mariana.*

At first glance, the disparity in the ways that the hematodochae of the two species are inflated and in the movements that they produce might seem surprising. On further consideration, however, it seems likely that the evolution of new sclerites and processes in spider pedipalps is often accompanied by new movements to employ these structures. Almost by definition, the use of a new process will involve new movements. Given that spider palps are driven by hydraulic pressure rather than intrinsie muscles (Huber 2004), differences in movements such as those documented here presumably result from differences in the forms of membranes that connect the selerites within the palp and the ways in which these membranes are folded and twisted. We hypothesize that the frequent evolutionarily rapid changes in sclerites in male spider palps are often accompanied by changes in the internal membranes of the palp, and that these membranes probably often have traits that would be useful characters for distinguishing species.

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Web construction of *Linothele macrothelifera* (Araneae: Dipluridae)

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Abstract. Direct behavioral observations, plus deductions made from studying the lines in recently built webs, showed that *Linothele macrothelifera* Strand 1908 lays swaths of lines in relatively stereotypic ways that differ during sheet web and tube construction. Sheet construction occurs in brief bursts interspersed with returns to the retreat. The legs are not used to manipulate lines; the spinnerets attach lines to the substrate and are probably used as sense organs. Asymmetrical use of the spinnerets during sheet construction results in an increase in the variety of orientations of lines in the sheet.

Keywords: Mygalomorph, sheet web construction behavior

Spiders in the family Dipluridae build some of the most elaborate prey capture webs among mygalomorph spiders (Coyle 1986). In the subfamilies Ischnothelinae and Euagrinae, several species build complex arrays of numerous short tunnels that connect multiple small sheets and that mostly capture ambulatory prey (Coyle 1986, 1988, 1995; Coyle & Ketner 1990). Some species in the subfamily Diplurinae, including species in the genera *Linothele, Trechona* and *Diplura,* construct a single large horizontal sheet with a tubular retreat. Some of these sheets are suspended in the air many cm above the ground, and have tangles that extend up to a meter or more above the sheet, while others are built on the surface of the leaf litter or some other substrate (Coyle 1986; Paz 1988; Viera et al. 2007). It appears that other than the brief mention by Paz (1988) of the behavior of *L. megatheloides* Paz & Raven 1990, nothing is known regarding the behavior patterns used by diplurids to build their webs.

This note reports observations of the building behavior of *Linothele macrothelifer* Strand 1908, which builds sheet webs on the surface of forest leaf litter. This species, as is typical of non-orb weaving spiders in general, adds lines to its webs on successive nights. Our observations make use of the technique of damaging webs in the field and then observing newly constructed replacement webs, whose more sparse lines facilitate determination of patterns in the spider's building behavior (e.g., Eberhard 1987; Benjamin & Zschokke 2003; Lopardo & Ramirez 2007).

METHODS

We made field observations on 1–4 December, 2011, near the end of the rainy season, at the Reserva Forestal de Yotoco (03°51′50″N, 76°26′17″W), a 550 ha patch of subtropical wet forest (Florez 1996), between 1300 and 1700m AMSL in the Western Cordillera of the Andes near Buga, Colombia. Sheet web construction behavior of one adult female was recorded using a SONY DCR-TRV50 video camera equipped with +7 close up lenses and infrared illumination. Individual lines emerging from her spinnerets were visible in some frames due to occasionally favorable angles of illumination. We collected portions of webs on small cardboard frames coated with double-sided adhesive tape, taking care to avoid including lines of other webs (e.g., of ochyroceratids) that were often built near the diplurid webs. Photographs of new webs were obtained by destroying sheets (leaving the tunnel mouth intact) in the afternoon and then coating webs with talcum powder the following morning. We include multiple web photographs because webs varied substantially in some respects (e.g., Figs. 1 & 2). Not all spiders whose webs were observed in the field were collected; we judged them to be mature females on the basis of the sizes of the spiders and their tunnels.

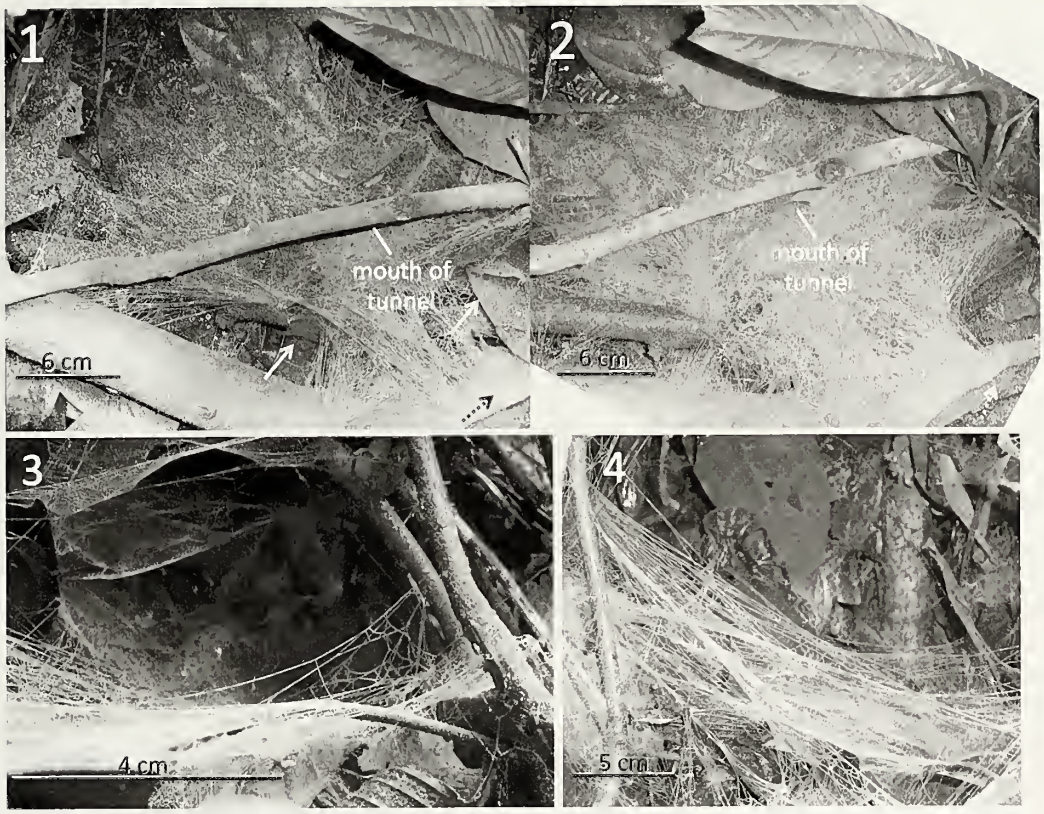
We made further observations of sheet web and tube construction in eaptivity by two other adult females by eovering the bottom of each of two 30 × 20 cm terraria with moist earth and creating tubular retreat eavities by inserting one finger. Video recordings were made from above with a digital Canon PowerShot A800 camera.

Means are followed by ± 1 SD. Because of the small samples, they are meant only to provide general descriptions of magnitudes, rather than to characterize the behavior of this species.

RESULTS

We followed the webs of 12 individuals of *L. macrothelifera* over the course of 2–4 days. All consisted of a horizontal sheet extending from the edge of a tunnel, and were attached to the upper surfaces of leaves and twigs in the leaf litter (Figs. 1–4). The sheets were not perfectly flat, but followed the general contours of the objeets in the litter. Usually nearly all lines formed a single sheet (Fig. 3), but some webs were somewhat multi-planar (Fig. 4). The individual lines in the sheets were relatively thin, and many were damaged or at least severely disorganized by the relatively moderate rains that fell daily (Fig. 5). In contrast, the walls of the tubular silk retreats were more dense and more protected, and persisted after rains. Thus the spiders largely rebuilt their sheets but not their tubes every evening following a rain. There were often large drops of water trapped in the complex, multilayered web near the mouth of the retreat, as also reported for *L. megatheloides* (Paz 1988).

Sheet construction behavior.—Spiders in the field were out of sight in their silk tunnels during the day, and came to the mouth of the tunnel about 18:00–18:30 to rest motionless, facing outward. Web construction in the field occurred in



Figures 1–4.—Webs of *L. macrothelifera*. 1 & 2. Dorsal views (from slightly different perspectives) of "replacement" webs built on two successive nights by the same spider at the same site after the web was removed the previous day. Solid white arrows in 1 mark empty areas that were covered by the sheet built on the second night (2); dashed arrows at the lower right mark areas with similar arrays of attachments to the substrate in both webs; 3. Lateral view of a replacement web that was nearly strictly planar, though sloping upward somewhat at the left rear (the web at the very top of the photo belonged to a different spider); 4. lateral view of a replacement web that was not strictly planar, with bands of silk in multiple dimensions.

bursts, at intervals on the order of 30–60 min when the spider made brief forays away from the tunnel mouth to lay lines. Multiple lines apparently emerged from all three spinneret segments of both posterior lateral (PL) spinnerets throughout each foray. In some cases the angles of new lines captured in video recordings indicated that they were attached at the last site at which the spinnerets had tapped or swept across the substrate, so tapping and sweeping motions are assumed to have resulted in attachments in the descriptions below. The mean number of attachments/foray in the field by sweeping a PL spinneret against the substrate (below) was 10 ± 10 (n = 7). Our observations of other spiders that were visited repeatedly on two other nights and of the two spiders in captivity also showed that the spiders added to their webs only in short bursts, followed by periods of immobility at the tunnel mouth facing outward.

Figure 5.—Dorsal view of the edge of a web that had been rained on but destroyed the previous day. On left are sparse, disorganized, presumably older lines, and on the right are denser bands of parallel, presumably newer lines.

Sheet construction was relatively stereotypic in several respects. The spider we recorded in the field produced a swath of lines from both of her long PL spinnerets throughout each foray away from her retreat. Lines in this swath were probably initiated by attaching to the walls or mouth of the tube as the spider began a foray. After moving more or less directly from her retreat to the edge of the web (or what would be the edge), the spider swung her abdomen laterally and extended the PL spinneret laterally on the side toward which she had swung her abdomen (Fig. 6a; mean = 24 ± 11°; maximum 45° in 56 cases). At the apogee of the lateral movement of her abdomen, this spinneret swept across the substrate, apparently attaching the swath of silk lines it was producing. During a sweep, the spinneret was lowered and it appeared that all three segments

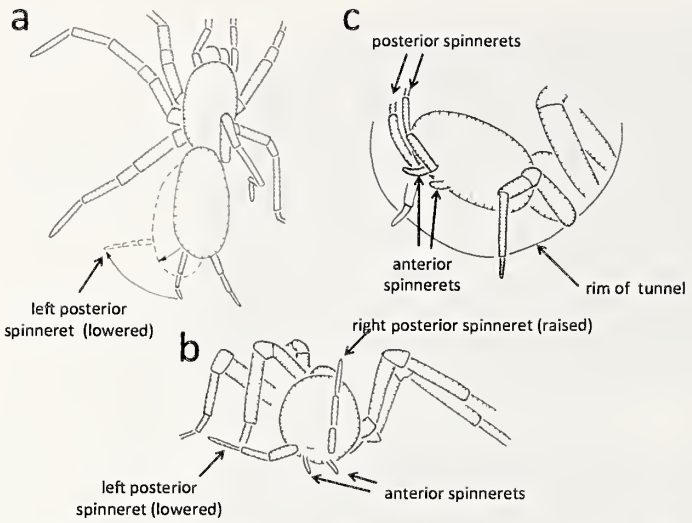


Figure 6a–c.—Drawings of construction behavior. a & b. Attachment behavior during sheet construction. a. In dorsal view, the left PL spinneret is directed laterally, while the abdomen is swung laterally and posteriorly to make an attachment; the right PL spinneret remains extended but does not touch the substrate (dotted lines follow solid lines by 1.70 s); b. in posterior view, the anterior spinnerets are lowered and are visible and the right PL spinneret is directed dorsally; c. in posterior-lateral view, the anterior spinnerets are lowered during tunnel construction to make simultaneous attachments by both PL spinnerets.

contacted the substrate (Fig. 6b). Attachments to objects in the leaf litter and to sheets of lines already in place were produced by similar swinging movements of the PL spinnerets and the abdomen.

Usually (46 of 56 cases), successive attachments during sheet construetion were to the same side. In two cases with a favorable angle of view, the anterior lateral (AL) spinnerets were visible and were also lowered to the sheet (Fig. 6b). Often the spider turned her cephalothorax slightly to the side opposite that to which she swung her abdomen; for instance, the spider's cephalothorax turned to the right as she swung her abdomen to the left and extended her left PL spinneret laterally.

Meanwhile the other PL spinneret usually stayed out of contact with the substrate (74.1% of 54 cases in which this detail could be seen). Often (44.4% of these 54 cases) it was directed dorsally (Fig. 6b). The lines emerging from this spinneret were sometimes not attached until the spider later swung her abdomen to the opposite side and lowered this spinneret and extended it laterally, or until she returned to her tunnel. The spider made up to ten consecutive attachments with one spinneret before attaching with the other. The spider usually directed both posterior lateral spinnerets dorsally while walking between attachment points, thus elevating the swaths of lines she was laying above the sheet and above leaves and twigs in the litter. The lines from the two PL spinnerets were thus often attached at different sites, the swaths of lines from the two spinnerets were often laid in different directions, and the lines laid from the less active PL spinneret were sometimes slack.

In no case did any leg hold any line that was being produced or to which the spider was attaching. Nor did legs tap as if locating potential attachment sites. Attachments were usually made to sites that had not been contacted previously by any legs (88.2% of 51 cases in which this detail could be seen). Instead it appeared that the spider used her long PL spinnerets as sense organs, and that their sweeping movements informed her of the presence of nearby objects.

The spider always made several attachments to the substrate during a foray away from the tunnel, but eventually returned to it by a more or less direct path. On arriving at her retreat, she attached several times in quick succession with her spinnerets directed more or less posteriorly (simultaneously with both spinnerets in five of seven cases) (Fig. 6c), then went inside and turned around to face outward.

All of these details were similar in a recording of sheet building by a spider in captivity (except that it was not possible to see either the lines emerging from her spinnerets or the positions of her anterior spinnerets). The spider that was recorded building a sheet in the field paused and struck through the sheet at a site where one of her tarsi had just dislodged a lump of earth that rolled below the sheet, raising the possibility that the sheets may be used to capture prey walking below the sheet as well as on it.

Tunnel construction.—Tunnel construction was seen only in captivity (n = 2 spiders). The movements differed sharply from those used to build sheets. The two PL spinnerets were usually both extended more or less directly rearward, and both touched the substrate simultaneously and repeatedly in close succession along their basal segments and at least sometimes also more distally. In some cases it appeared that the AL spinnerets were also lowered and made repeated contact with the tunnel wall (Fig. 6c). Spinneret contact with the substrate resulted mostly from an anterior-posterior rocking movement of the entire body, combined with minor ventrally directed movements of the spinnerets themselves. The PL spinnerets moved slightly apart as the spider rocked posteriorly, and then moved slightly together as she rocked forward. Contacts with the wall of the tunnel in successive taps or thrusts with the spinnerets were frequently closely spaced. As in sheet construction, the spider never used her legs to manipulate either the lines being laid or the lines to which they were being attached.

Tunnel construction resulted in silk being spun across the tunnel entrance. At the end of a bout of spinning, the spider broke through this sheet at the mouth. She inserted her anteriorly extended legs I and II between lines there, then moved the legs laterally and stepped forward, thereby forcing her body through the sheet.

Web photographs.—Patterns of lines captured in web photographs confirmed and extended the direct observations of sheet construction behavior. Replacement sheets were mostly attached to the substrate near their edges. Some replacement sheets were attached at only a few points (as few as about 12), with multiple lines fanning out from each of the attachment sites (Figs. 1, 2, 5). Attachment sites with lines fanning out from them were generally at the edge of the sheet, and there were seldom any points with lines fanning out from them in the central area of a sheet. These patterns confirmed the behavioral observation that attaehments were concentrated near the edges of the sheet. One attachment point at an especially sparse edge of a web had approximately 20 lines

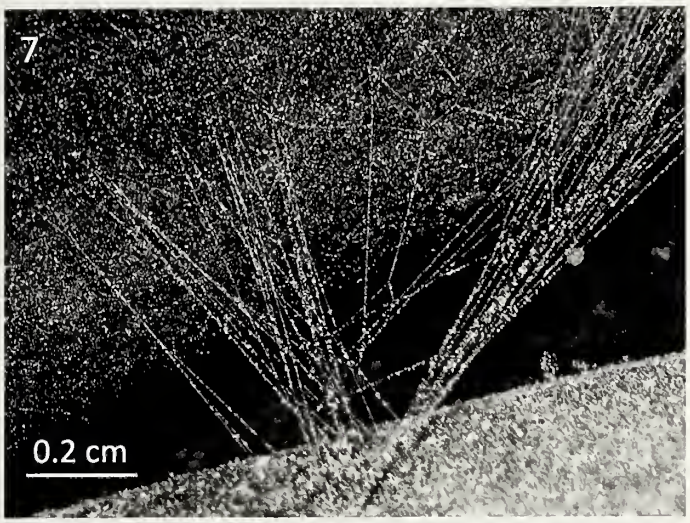


Figure 7.—Approximately dorsal view of a site to which the spider apparently made only one sweep with one spinneret, depositing approximately 20 lines.

running to it and an equal number away from it (Fig. 7); another swath of parallel lines in an especially sparse web also had approximately 20 lines (Fig. 8). Thus a single spinneret probably produced on the order of 20 lines. Some peripheral attachment points in replacement webs had many lines radiating from them (Figs. 1, 2, 5, 8), suggesting multiple visits and indicating that there were substantial variations in the directions from which the spider arrived at the point and in which she moved when leaving it. One "older" web (which had been destroyed the day before) had a highly reinforced band of lines running from the retreat and between four adjacent peripheral attachment sites, suggesting that the spider had repeatedly left the retreat and travelled from one to the next to the next of these sites (Fig. 5).

Two replacement webs that were built on successive nights by the same spider at the same site showed differences in the arrangements of the lines (Figs. 1 & 2). Thus, building movements were not highly stereotypic, even for webs at the same site.

Web samples under the microscope.—We examined samples of the sheets of four webs under the microscope. The lines clearly had multiple diameters (Figs. 9a & 9b). Most lines were relatively straight, and in only a few cases did lines appear to adhere to each other and exert tension (as indicated when one line pulled another line into an angle $< 180°$; Fig. 9c); there was no sign of substantial thickenings such as attachment discs at such sites (Fig. 9c). There were some complex arrangements, however, such as cables of multiple lines, extensive lax lines, and apparent adhesions between loose lines (Fig. 9d).

DISCUSSION

We made only fragmentary observations, and further studies are needed. Nevertheless, the combination of direct observations of behavior and deductions from web photos are sufficient to clarify some basic points. The standard pattern of movements used by *L. macrothelifera* to build a sheet web appears to be to lay a swath of lines while the spider walks, to use asymmetrical movements of the two long PL spinnerets to attach the lines at several points near the periphery of the web, and then to return more or less directly to the retreat, laying

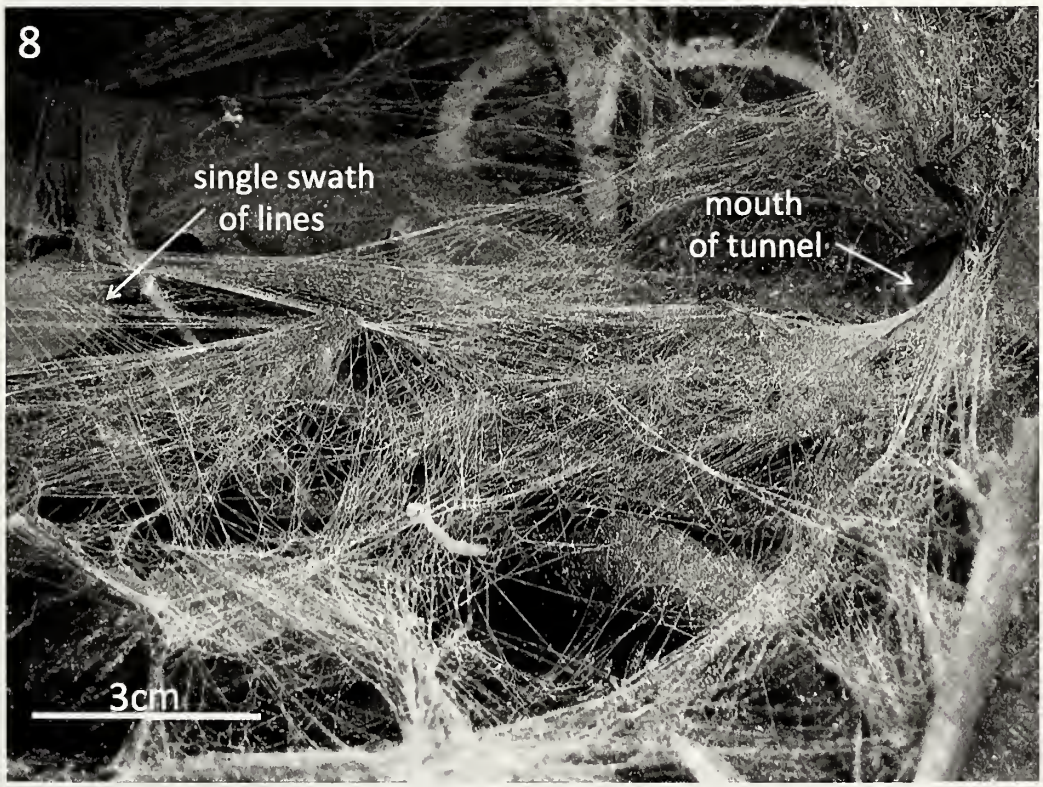


Figure 8.—Dorsolateral view of a relatively sparse replacement web, showing how bands of parallel lines were attached to upward projecting objects in the leaf litter. A single swath of about 18 parallel lines that was laid on top of other lines is visible at the left.

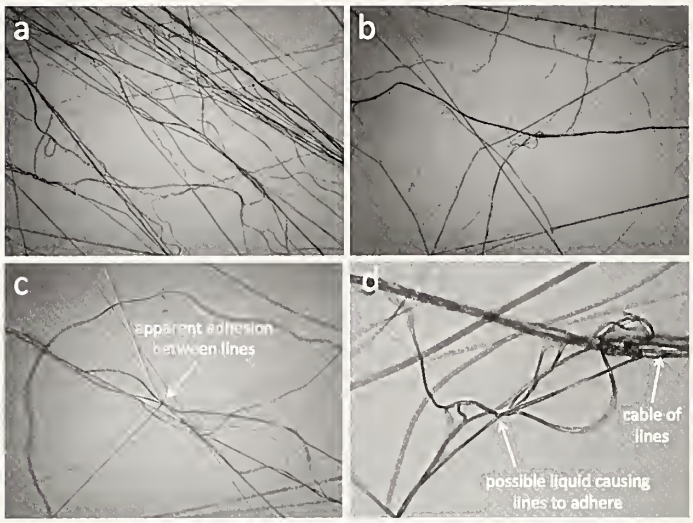


Figure 9.—Lines in a sheet seen under a compound microscope. a & b. Typical arrays of many more or less parallel lines of different diameters, with some lines loose and others tight; c. an unusual site where two lines apparently adhered to and deflected each other; d. a cable of multiple lines and a possible small accumulation of liquid at a site where lines apparently adhered to each other.

further lines that are attached there. Even early excursions from the retreat reached the edge of the web (i.e., the web was not gradually extended outward away from the retreat). We saw no sign of the irregular forward, backward, and sideways movements mentioned by Paz (1988) for *L. megatheloides* (whose sheet is aerial, and not in contact with the leaf litter).

In contrast, tube construction by *L. macrothelifera* involved more synchronous and symmetrical movements of the PL spinnerets to produce more frequent, closely spaced attachments. The atypid *Sphodros rufipes* (Latreille 1829) also builds its tube using frequent, closely spaced attachments with her actively moving spinnerets (W.G. Eberhard unpubl. observ.).

A similar retreat-centered organization of web construction behavior also occurs in some sheet web spider such as the agelenid *Melpomene* sp. (Rojas 2011) and the theridiid *Parasteatoda tesselata* (Keyserling 1884) (Jörger & Eberhard 2006). Some other sheet web builders, in contrast, do not organize their sheet extension behavior around a central point; these include the pholcid *Modisimus gnatuso* (Eberhard 1992), and the linyphiids *Linyphia hortensis* Sundevall 1830 and *L. triangularis* (Clerck 1757) (Benjamin & Zschokke 2004) (and also possibly an unidentified ochyroceratid – M. Ramirez pers. comm.). In all of these other sheet-web groups, the spider first produces a skeleton web for the sheet and later fills in this skeleton. We saw no sign of this possibly derived pattern in *L. macrothelifera*.

One apparent inconsistency between the direct behavioral observations and the deductions from web photographs was that the spiders showed no behavioral indications of testing for or sensing the presence of lines and attachments of lines; nevertheless, the photographs clearly showed repeated attachments to particular supports (e.g., Figs. 5, 8, 9). Perhaps these sites were more elevated, or distinguishable in some other way that did not require any overt searching behavior other than waving the spinnerets.

Paz (1988) reported that the anterior spinnerets of *L. megatheloides* produced glue that fastened lines together, but gave no evidence to support this claim; we saw no discreet masses of material that attached lines to each other in *L. macrothelifera* webs. In some places very small amounts of liquid appeared to join lines (Fig. 9d). Mygalomorphs are thought to lack piriform glands or spigots that could glue lines together (Blackledge et al. 2009). It seems likely that the lines of *L. macrothelifera* were slightly wet when they emerged (as in the aciniform lines of labidognaths – see Eberhard 2011), and that this explains their adhesion to the substrate and to each other.

The legs of *L. macrothelifera* played little if any role in either locating attachment sites or manipulating silk lines during any stage of web construction. Instead the spider's long PL spinnerets seem to be used as sense organs to locate attachment sites. Perhaps the flexibility that is presumably provided by the widespread pseudosegmentation of the long terminal PL article in diplurids (Coyle 1995) enhances this function. The frequent asymmetry in the use of the PL spinnerets during sheet construction, with one kept raised while the other was lowered and swept across the substrate to make an attachment, resembles prey wrapping behavior by the theraphosid *Psalmopeus reduncus* (Karsch 1880) and several araneomorph species (Barrantes & Eberhard 2007). Presumably its function in diplurid sheet construction, as perhaps also in these other contexts, is to generate lines running in a greater number of different directions. Paz (1988) noted that the spinnerets of *L. megatheloides* moved with respect to each other and the long axis of the spider's body, but did not describe the patterns we report here. The atypid *S. rufipes* also laid lines with bobbing movements of the abdomen and no direct involvement of the legs (W. Eberhard unpubl.).

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SHORT COMMUNICATION

The rare large prey hypothesis for orb web evolution: a critique

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Abstract. Several recent studies emphasize, correctly, that the biomass of prey captured by an orb web is likely more important than the number of prey in driving the evolution of web designs. Using equations that estimate prey mass from the lengths of captured prey, one study concluded that rare, long-bodied prey contribute the large majority of energy obtained by orb weavers in general, and thus that the designs of orb webs should generally reflect selection favoring the capture of larger insects, especially the ability to absorb high-energy impacts. I show here that the weights of long prey have sometimes been seriously overestimated by these equations. In addition, the longer prey captured by spiders probably represent highly biased samples, in terms of both low weight/body length and low momentum/body weight, of the longer prey available in the environment, leading to overestimates of the kinetic energy that the orb needed to absorb to stop them. Deductions concerning how selection acts on orb designs that have been based on the prey captured are also flawed, because additional data on prey availabilities and prey escapes are needed to evaluate the possible gains and losses from different orb designs. Still another complication is that data on prey abundances in natural rather than altered environments are needed to understand past selection pressures that produced present-day web forms. I conclude that a dominant importance for rare large prey in orb web evolution has not been conclusively demonstrated to be a general rule for orb weavers. A more inclusive approach regarding orb functions is prudent, especially because many traits that improve some functions have opposite effects on others.

Keywords: Orb web evolution, rare large prey hypothesis

Orb webs perform multiple functions. These include intercepting prey, stopping them without breaking, retaining them until the spider can attack, transmitting prey vibrations to the spider, facilitating the spider's movements across the web, and others (Witt 1965; Blackledge et al. 2011). The traits that will improve some functions of an orb are likely to make it less able to perform others (Table 1), complicating understanding of orb evolution. In addition, some functions are very complex. For instance, retention is affected by the positioning of the glue droplets on the axial fiber, the mechanical properties of the axial fiber, the arrangements of the sticky lines, the surface of the prey, the prey's impact velocity (higher velocities make the line stickier), and the humidity of the environment (Blackledge et al. 2011). The optimum balance of web traits is likely to vary for different prey, for different body conditions of the spider, and in different environments (Table 1; Blackledge 2011). It has thus been difficult to determine the net advantage or disadvantage of particular orb traits. Recent emphasis on the possibility that rare, large prey dominate the nutritional payoffs to orb weavers (Venner & Casas 2005; Blackledge 2011) promises to simplify this problem by focusing on the abilities of orbs to promote the capture of large prey, thus justifying special emphasis on the stopping function (Sensenig et al. 2010). In this note I argue, however, that such a focus is not well justified by currently available data.

Prey weight: important but difficult to determine.—A spider's basic payoff from an orb is nutritional, and larger prey organisms offer greater nutritional reward than smaller prey organisms. Small prey, however, are generally more abundant than large prey, but of course they weigh less and have less digestible material per gram, due to their having relatively greater surface areas and hence a greater proportion of chitin. Thus the balance of benefits from having an orb designed to capture larger or smaller prey is not immediately obvious. Under some conditions, the relative frequencies of prey and the sizes of payoffs from prey of different sizes could result in the payoffs from rare, very large prey items to be so much greater than those from more common smaller prey that the smaller prey are nutritionally irrelevant for reproduction. The "rare large prey" hypothesis posits that this condition generally obtains for orb-weaving spiders. Other possible mixes of prey, such as higher numbers of medium-sized prey, or especially rare large prey, could result in a reduced relative importance for large prey (Nentwig 1985). The rare large prey hypothesis is important for understanding the evolution of spider webs, because it implies that natural selection consistently favors the designs of orbs and the physical properties of their lines that promote capture of especially large prey (see Sensenig et al. 2010; Blackledge 2011; Blackledge et al. 2011).

The rare large prey hypothesis greatly simplifies analyses of the possible evolution of orb web designs, because it allows one to navigate the complex array of tradeoffs between traits that can affect the many different possible functions of orbs (Table 1), and to focus on the ability of an orb to absorb high kinetic energy impacts without breaking. This opportunity was exploited recently in the important study by Sensenig et al. (2010) of 23 species in four families, where an orb's "performance" was defined as its ability to absorb high-energy impacts.

The validity of the rare large prey hypothesis depends on the masses of small and medium-sized prey being small enough, and/or their capture being rare enough that they make only relatively small contributions to the spider's dietary intake compared with the contributions of the rarer, large prey. Do these conditions occur in nature? In the most complete study to date, Venner & Casas (2005) found that large prey were indeed captured only rarely by spiders in the *Zygiella x-notata* (Clerck, 1757) population that they studied. They measured the lengths of all prey found in webs, and estimated their probable dry weights using a standard equation that converts insect length to dry weight (Schoener 1980). They made the assumption that a prey's dry weight is a good indicator of its contribution to the spider's nutritional intake. Although a previous control study showed that the dry weight equation of Schoener yielded only approximate indications of the amount of ingestible food for spiders (Tso & Severinghaus 1998), they increased the biological realism of their analysis by determining the maximum amount a spider could consume in a day (9.4 mg of dry weight) and held gains

Table 1.—This list of possible trade-offs in the advantages of alternative orb web designs illustrates the complex tangle of inter-relationships that probably exist between the advantages and disadvantages of different orb web designs. The supposition is made that the spider has only a finite amount of material resources that can be dedicated to alternative designs. The advantages and disadvantages marked with "*" are likely to be especially pronounced for relatively large prey. The list of trade-offs is undoubtedly incomplete.

Advantages	Disadvantages
Larger sticky spiral spacing and thus greater capture area	
More prey intercepted; Web less easily perceived by prey	*Reduced ability to stop and retain prey Reduced ability to survive environmental stresses such as wind Greater energy expended in building behavior
Nearer to an approximately vertical substrate[a]	
More difficult for prey to perceive web *Prey flying more slowly	Fewer interceptions (prey arrive from only one side)
Horizontal (rather than vertical)	
Less energy expended in construction Reduce loading by wind More rapid attack[c] Greater portion of web close to a prey-rich horizontal substrate (e.g., surface of water)[d]; Less distortion of web by spider's weight while building (more precise positions of lines) More equitable distribution of tensions from spider's weight at the hub (greater mechanical stability)	Reduced prey interception[b]; *Prey encounter fewer lines as they struggle free and fall from the web
Thinner lines	
Longer lines, thus increased area covered (improved interception) and/or *greater density of lines (improved retention)	*Reduced ability to stop and retain prey
Web less visible for prey	Reduced ability to survive environmental stresses Reduced ability to support spider
Greater amount adhesive on sticky lines	
*Increased retention times Increase prey attraction to "sparkly" droplets[e]	Reduced sticky line length (reduce numbers of prey intercepted Web easier for prey to perceive
Tighter web	
Better able to survive in wind because it flaps less Less distorted less by spider's weight during construction (more precise positions of lines) Improved transmission of vibrations[h]	*Less able to stop and retain prey[f] Less web movement in light wind[g]
More radii	
*Better able to stop prey; Improved resistance to environmental stress such as wind	Increased silk cost, or fewer interceptions because web smaller; Orb more easily perceived visually by prey[i] Greater energy expended in building web (at least in radius construction)
Larger spaces between sticky spiral loops near the edge	
Increased interception near the edge	*Reduced ability to stop and retain prey near the edge

[a] More or less planar substrates could include, for instance, a tree trunk or a large leaf.

[b] This prediction makes the assumption that the flight paths of prey are mostly horizontal. This trend in flight paths may be surprisingly small, however. When identical artificial sticky traps consisting of lines strung in frames made of strips of aluminum about 2 cm wide (Eberhard 1977) were hung vertically and horizontally at the same height in a barbed-wire fence across a uniform, open field, the horizontal traps captured nearly a third of the total number of insects captured in vertical traps, despite the fact that no prey travelling perfectly horizontally would have been intercepted by any of the sticky lines in the horizontal traps (Chacón & Eberhard 1980). In sites that are less open, non-horizontal flight paths (and thus interceptions by horizontal orbs) may be even more common.

[c] This is expected at least comparing prey impacts to the side of and above the spider in vertical orbs.

[d] Documented by Buskirk (1975) near stream surfaces in Costa Rica for *Metabus gravidus*; a similar trend was clear in artificial traps (Eberhard 1977) that were hung just above a stream near Cali, Colombia (el. 1050 m) (W. Eberhard, unpublished).

[e] Only a single species of prey (a stingless bee) was tested for attraction of "sparkle" (Craig & Freeman 1991).

[f] The hypothesis that lower tension increases prey retention is supported by the independently evolved active reduction of web tension by the spider when prey hit the web in *Hyptiotes, Epeirotypus, Micrathena,* and *Wagneriana* (for a discussion of the physics involved, see Craig 2003).

[g] Support for the hypothesis that flapping movements of webs in the wind play an important role in increasing the web's abilities to intercept prey (the "encounter model" of Craig 1986) was based on measurements that probably overestimated web movements (the webs were loaded with cornstarch), so the possible importance of this factor is not certain.

[h] This is surely true in the extreme case comparing lines under tension with slack lines, which scarcely transmit the longitudinal vibrations that are used by spiders (see Landolfa & Barth 1996).

[i] Craig & Freeman (1991) state, probably correctly, that the sticky spiral is largely responsible for determining an orb's visibility; but, at least to the human eye, the glint of radii when they are illuminated at an appropriate angle is also visible.

constant for prey with estimated dry weights above this value. They also measured the weights of egg masses, of females when they laid egg masses, and of spiders when they died of starvation, the rate of basal metabolism and assimilation of prey. Using these data, Venner & Casas (2005) calculated that without the rare captures of large prey, the spiders could not reproduce.

There are several complications in this study. The largest prey were reported to be "mainly" crane flies; these flies have long thin bodies, making the equation used to estimate a prey's weight on the basis of its length give overestimates of their weights. The error can be quite large. I measured and weighed 11 individuals of a large species of tipulid in Baton Rouge, LA (mean length = 14.4±3.1mm), and found that the dry weight calculated from the equation that Venner & Casas used, $W = 0.024 + L^{2.35}$, was on average 65 times higher than their measured dry weight (mean 10.2±7.1 mg). The use by Venner & Casas of an upper limit on prey consumption (which was also obtained when tipulids were the prey – S. Venner pers. comm.) probably reduced this type of error somewhat (to an unknown extent) in their estimates. This likely imprecision calls into question (but does not disprove) the claim that payoffs from large rare prey were determinant for reproduction in *Z. x-notata* (Venner & Casas 2005). Recalculations that correct for different body designs of prey or direct measurements of prey weights are needed. In fact, Schoener (1980:106) warned specifically against just this kind of problem ("Workers who use length-weight equations to estimate overall biomass are cautioned not to lump insects having markedly different body proportions into the same regression").

Biases in captured prey and their flight speeds.—Setting aside the unusual body design of crane flies, there is a more general problem with using an equation that gives a best general estimate of weight in general samples of insects to study selection on orb webs on the basis of the prey that they capture. This is because in general the largest prey captured (tipulids and others) are likely to be a highly biased subset of the large prey in the habitat, favoring prey with low weights with respect to their lengths, and they are thus especially likely not to follow the Schoener relationship. The species whose impacts involved the least energy and were thus most likely to fall prey to the spider were most likely to be captured (the heavier, faster prey of that same length will tend to escape due to their higher impact energies). Of the especially long prey in the environment, only the lighter ones are likely to be detained; of the shorter prey, in contrast, both relatively light and relatively heavy individuals are likely to be detained. In other words, the Schoener equation is designed to describe length-weight relations in a random sample of insects. But the prey captured by orb weavers are not expected to be a random sample (the larger ones are expected to have relatively low weights in relation to their lengths). Thus the equation is expected to be inappropriate for this subsample. This bias is likely to be especially strong for the largest, most difficult prey for the orb weaver to capture.

Still another problem in extrapolating from the Venner & Casas study is that a prey's flight speed also affects its momentum (and thus the energy the orb must absorb to stop it) (see Blackledge & Zevenberg 2006 for examples of differences in the momentum of different prey species). For instance, crane flies are atypical among insects with respect to flight speeds: they generally fly slowly, and have long, weak legs (Borror et al. 1989), making them especially easy to stop with an orb. Their slow, tentative flight constrasts, for example, with that of many flying beetles or grasshoppers of similar body lengths. In addition, the *Z. x-notata* webs in the Venner & Casas study were nearly all built close to and parallel to the windows of a building (S. Venner pers. comm.), making it likely that many of the tipulids, as well as other large prey that the spiders in this study captured, were flying relatively slowly. From my own incidental observations of the flight of several species of crane flies, I would guess that many of the tipulids that were capturd by the *Z. x-notata* orbs had "bounced" repeatedly against the windows; and that, as occurred in trials in captivity with the similarly long-legged and tentative flier *Hyalobittacus* sp. (Blackledge & Zevenberg 2006), most escapes from orbs were due to failures to retain the tipulids (and perhaps other insects) after their momentum had been absorbed by the webs, not to failures to stop them. The important general point here is that the impact of a large prey with an orb does not necessarily involve large momentum. Therefore, finding occasional large prey captured by orb weavers does not necessarily indicate that their orbs successfully resisted high-energy impacts.

In sum, these considerations do not argue against the validity of the important insight of Venner and Casas that prey mass is much more important than prey numbers to the survival and reproduction of orb weaving spiders. But the biomass of prey captured by *Z. x-notata* may not have been as heavily biased toward larger prey as they calculated; and, even accepting their biomass calculations, the capture of large prey clearly did not imply that *Z. x-notata* orbs successfully resisted high-energy impacts.

General trends in selection on orb webs?—Blackledge (2011) followed up the Venner & Casas (2005) study by asking whether or not it is a general rule that the mix of different-sized prey for different orb-weaver species is such that the payoff from small and intermediate-sized prey tends to be so small that natural selection consistently favors traits designed to capture larger, rarer prey (I will call this a "Venner & Casas mix"). He concluded, after classifying prey as "large" if they were >2/3 of the spider's body length and applying the same Schoener equation (again indiscriminantly to all prey) in a meta-analysis of 38 studies of the prey of 31 species in 18 genera of orb weavers, that the answer is yes: "The 'rare, large prey' hypothesis thus can apparently be generalized across orb spiders." (Blackledge 2011:205, abstract). He argued that this means that "... the latter metric (the ability to stop and retain insects rather than intercept them) is more likely to play a decisive role in determining fitness" (p. 209).

Two of the problems noted in the previous paragraphs are also relevant for this Blackledge study: prey weight cannot be reliably estimated from prey length, especially in a small, likely biased sample of the longer species captured by an orb weaver; and the capture of a large prey by an orb weaver does not reliably imply that its orb resisted high-energy impact. Even Blackledge's worst case equation to estimate prey weight, with an exponent of 2 for L, overestimates the dry weights of the tipulids I measured by >2500%. The correction used by Venner & Casas (2005) for the upper limit on the amount of food a spider can consume in one day was not applied in Blackledge's study, accentuating the potential for overestimates of the importance of longer prey.

In addition, there are questions concerning the evolutionary realism of the 38 studies. The list of habitats includes "orchards", "fields", "buildings", and "cotton field". The existence (or lack of existence) of a Venner & Casas mix in these types of unnatural settings has no logical implications for whether Venner & Casas mixes occurred in the natural settings where the orb designs of these species evolved.

The most basic problem with this study concerns the translation of data on prey captures into conclusions about how natural selection acts on orb web designs. I believe that the data used by Blackledge (2011) are not equal to the task of drawing convincing conclusions. The location of the optimum point in the tradeoff between interception and stopping is not expected to depend only on the frequencies with which large and medium prey were captured, but also on additional variables, including their relative abundances in the environment, their relative numbers of encounters with orbs, and their relative frequencies of escapes. These additional factors will determine the possible gains or losses to be expected from changes in web design. Simply counting up the prey captured and estimating their weights is not enough. Nor will it suffice to combine weights of captured prey with data on the numbers and weights of prey of different sizes in the environment, because attempts to assay the prey

that are actually available to an orb weaver have had serious problems, and are generally unreliable (Castillo & Eberhard 1983; Eberhard 1990). Thus the data in Blackledge's meta-analysis, and indeed those in most if not all studies of prey captured by orb-weavers in nature, are not sufficient to test ideas regarding selection on different orb designs.

Evidence from web designs.—If the rare large prey hypothesis is generally true, the implication is that selection consistently favors orb designs that are better able to stop high-energy prey. But the web designs of a variety of species do not fit this prediction. The webs of the theridiosomatid genus *Olgunius* (Coddington 1986), of *Cyrtarachne* and its relatives *Pasilobus* and *Poecilopachys* (Shinkai 1979; Carran & Miyashita 2000; Robinson & Robinson 1975; Clyne 1972), and of *Meta reticuloides* Yaginuma, 1958 (Shinkai 1969) all have very low numbers of espeeially widely spaced radii and sticky spiral loops. The flimsy, open-meshed orbs of species in the large genus *Tetragnatha* also seem poorly designed for stopping high-energy prey (Comstock 1948; Kaston 1948; Shinkai 1979; Gillespie 1987). In at least one species, *T. kaestneri* (Crome, 1954), field observations of prey emphasized the importance of relatively small, low-energy flies (Chironomidae) (Crome 1954). In another species, *T. lauta* Yaginuma, 1959, an even sparser array of radii and sticky loops has evolved (Shinkai 1988).

Another, more taxonomieally widespread example also calls into question the supposedly dominant role of the stopping function and, more generally, the trumping effect of stopping plus retention over interception (Blackledge et al. 2011). This concerns the pattern of sticky spiral spacing within the webs of many orb weavers. It is common for the spaces between sticky spiral loops to be larger in the outer portions of an orb than near the hub in Araneidae, Tetragnathidae, Theridiosomatidae, and Uloboridae (LeGuelte 1966; Herberstein & Heiling 1998; see also photos in Comstock 1948; Kaston 1948; Shinkai 1979; Coddington 1986; Shear 1986; Kuntner et al. 2008). That is, in just those portions of these orbs in which the orb's ability to stop high-energy prey with its radii is the lowest, the sticky spiral loops are placed farther apart, further reducing the web's abilities to stop and retain high-energy prey, but increasing its ability to intercept them. This raises the possibility of different balances between different functions in different parts of a single orb. Although it is possible that intra-orb differences (if they exist) in the properties of sticky and non-sticky lines (e.g., Crews & Opell 2006; Herberstein &Tso 2011; Blackledge et al. 2011) may complicate interpretations, this pattern does not seem likely to be the result of selection favoring stopping and retaining high-energy prey at the expense of interception.

Conclusions.—In sum, I believe that Blackledge's conclusion that the rare, large prey hypothesis is "... generalizeable across orb spiders" is overly ambitious. Where does this leave us regarding models concerning the evolution of orb webs? I am convinced of the importance of the insight of Venner & Casas that the biologically important variable for a spider is not the number of prey it captures, but the amount of resources that it receives from these prey. This insight renders irrelevant much previous work on orb webs that emphasized prey numbers, including some of my own (Castillo & Eberhard 1983; Eberhard 1986). But extrapolating from this insight to gain an understanding of selection on orb web designs is not simple. Constructing models based on a particular function such as stopping prey (Sensenig et al. 2011) can be useful as explorations of that particular function. And of course constructing models always involves making simplifying assumptions; there is no magic degree of biological realism that a model must fulfill. But omission of variables that are known to be important, especially when those variables have effects that run counter to a key variable such as the stopping function in a hypothesis (see Table 1), can reduce the likely usefulness of a model for understanding the real world.

The major eonclusion of this note is that until better confirmations of the rare large prey hypothesis are available, it will be wise to keep in mind the multiple functions of orbs, and avoid emphasizing only the stopping function to the exclusion of all others, because of the perceived importance of large prey (Sensenig et al. 2011). This does not mean that there may not be species in which the importance of the stopping function trumps that of all others (I suspect that such cases probably exist). Nor do I wish to imply that it is not useful to explore traits affecting this (or any other) particular function. But the importance of studies that attempt to understand the evolution of orb web designs in general is likely to be compromised if their emphases are too narrow.

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SHORT COMMUNICATION

What is the function of 'pre-dispersal' behavior in juvenile social spiders (*Stegodyphus dumicola*: Eresidae)?

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Abstract. Bridging and ballooning dispersal in spiders are preceded by "tiptoe" behavior, in which the spider stands on the tips of its eight tarsi, with the legs extended downward and the abdomen raised, and releases one or more silk lines. The occurrence of tiptoe behavior has been used in experiments to indicate a propensity to initiate dispersal. Juvenile social spiders *Stegodyphus dumicola* Pocock 1898 (Eresidae) exhibited tiptoe behavior while walking along the upper strands of the capture web at night. Simultaneously, they released long silk lines that streamed upward. These behaviors were not followed by dispersal. In wind-tunnel tests we found that tiptoe behavior increased with time during the evening activity period and occurred with higher frequency in small individuals. We suggest that tiptoe behavior in juveniles of *S. dumicola* is not associated with dispersal, but is more likely a component of web-building.

Keywords: Ballooning, bridging, tiptoe behavior, web-building, wind tunnel

Many spiders use ballooning for long-distance dispersal (Foelix 2011). Aerial dispersal is particularly common in araneomorphs, and occurs more often in juveniles than in adults, although in small species such as many linyphiids, adults disperse in this manner. Aerial dispersal is typically initiated by "tiptoeing": the spider stands on the tips of its eight tarsi, with the legs extended and the abdomen raised, and releases one or more silk lines. The immediate function of tiptoe behavior is to elevate the spider's spinnerets, thereby increasing the velocity of air flowing past them and facilitating the release of silk (Suter 1999; Foelix 2011). When enough upward force is generated on the silk, the spider releases its grip on the substrate and becomes airborne (Suter 1991, 1999). Aerial dispersal can also be initiated when the spider descends on a dragline and releases a free-ended line and is eventually carried off on the line (Eberhard 1987).

Tiptoe behavior also occurs in the context of bridging or rappelling, a form of short-range dispersal (Eberhard 1987), in which the end of the released line becomes snagged on an object and the spider then uses the line as a bridge. Numerous experimental studies of dispersal conducted in wind tunnels have used tiptoe behavior as an indication of the propensity to initiate ballooning or bridging dispersal, and have referred to it as "pre-dispersal behavior" (e.g., Weyman et al. 2002; Weyman 1995; Bonte et al. 2003; Bonte et al. 2009; Larrivée & Buddle 2011).

The release of free-ended lines, however, is not restricted to dispersal: it may occur in the initial stages of web construction as well. The first stage of web building involves establishment of a bridge thread between two separated points (Foelix 2011). Bridging behavior during web construction may be initiated when the spider releases a silk line, which drifts with the air current, becomes snagged and is then reinforced. Release of the bridging line was described as similar to "spontaneous" initiation of lines (Eberhard 1987), when the spider stood with its abdomen lifted and silk was drawn out in the breeze (S. Zschokke personal communication). Thus, tiptoe behavior may be a reasonable indicator of dispersal in species that do not build webs, but is perhaps not an unambiguous indicator in web-building species.

During observations of colonies of the social spider, *Stegodyphus dumicola* Pocock 1898 (Eresidae) in Namibia, we saw juveniles adopting the tiptoe stance on upper strands of the capture web during the start of their activity period at dusk. Under the assumption that tiptoe behavior indicates dispersal propensity, we expected to see these spiders lift off the web or rappel to nearby vegetation. Instead, the spiders moved along the web and alternated between laying down dragline silk on the web and adopting tiptoe behavior. This observation led us to question the function of tiptoe behavior in juvenile *S. dumicola*. To obtain insight into the contexts in which this behavior occurs and its possible function, we observed juveniles on the web, and we collected individuals from webs during their activity period and tested their behavior in a wind tunnel.

We investigated colonies of *S. dumicola* at Uisib farm (19°33′S, 17°13′E), near Otavi, in northern Namibia during January 2011 (southern hemisphere summer). A colony consists of a nest of dense silk and leaves, and one or more loosely constructed, cribellate-silk sheet webs radiating out of the nest in different planes. A nest may contain a few to several hundred individuals (Seibt and Wickler 1988). Colonies increase in size by inbreeding within the nest and by retention of juveniles in the colony (Lubin & Bilde 2007).

Eleven colonies were located in grassy roadside vegetation. Nests were attached to shrubs or small trees at a height of 0.2–1.5 m and contained mostly juveniles and a few subadult and adult males. We observed spiders in two medium-sized colonies on shrubs separated by 6 m (nest dimensions: 0.25–0.3 m length and ca. 0.2 m wide) and one large colony (nest ca. 0.5 m diameter) located in a tree about 100 m from the other two colonies. All nests had extensive capture webs that occasionally were damaged by heavy rains and livestock. We observed the behavior of 5–10 spiders per colony, chosen haphazardly, for up to 30 min per colony, between 17:00 and 22:00 local time (GMT+1), when spiders were active in web repair and prey capture. We observed the first two colonies during five nights between 11–20 January 2011, and the third colony on a single night. We did not quantify the observations and our notes serve only to describe the behaviors as we observed them in nature.

In testing for pre-dispersal behaviors, we adopted a technique similar to that used by other researchers (e.g., Weyman 1995). The wind tunnel had 0.3 × 0.6-m glass sides and a fan at one end. The fan drew air through the chamber at velocities that could be regulated both by adjusting fan speed and by opening or closing vents along the seal between the fan and the chamber. We did not attempt to reduce turbulence in the air flowing into the 0.3 × 0.3 m opening of the wind tunnel. In our tests, wind speed varied between 0.5 and 0.7 m/s, measured with a digital anemometer (Kestrel 4000, KestrelMeters.com).

The wind tunnel was suspended above a table at a ~45° angle with the surface of the table. On the table, at the lower end of the wind tunnel, we placed a bowl filled with water, with a 0.3 m long wooden dowel fixed at a 45° angle in a plasticine base in the center of the bowl, protruding into the wind tunnel. Some silk from the nest being tested was wrapped around the upper part of the dowel. The water prevented the escape of the spider.

We collected juvenile spiders (total body length, mean ± SD = 7.3 ± 0.9 mm, range: 4.6–9.2 mm, n = 79) for the experiments 0.5–2 h before testing (during 18:30–21:50) from the three colonies noted above. The juveniles were likely to be females, as subadult and adult males were already present in all three colonies; males mature before females, and the colony sex ratio is highly female biased (Henschel et al. 1995). We used soft forceps or a vial to collect groups of juveniles active in web-building or groups of juveniles on prey. We kept the spiders in these groups, classified according to source nest and activity on the web. Each spider was released onto the dowel individually, and we observed the behavior in the wind tunnel for up to 5 min using weak headlamp lighting. Each spider was tested only once. We segregated the spiders into two groups after testing according to whether or not they exhibited tiptoe behavior within 5 min of being placed on the dowel and measured them with calipers (total body length and prosoma width, measured with digital calipers to 0.1 mm). We conducted the tests during January 11–20, 2011, between 19:30–22:40. Air temperature during the tests was 20.5–25.2° C, and late afternoon rains ensured high humidity.

We documented tiptoe behavior using a DSLR camera (Nikon D200) fitted with a 105-mm macro lens and two off-camera flashes, one to illuminate the spider and the other providing strong backlighting to make the spider's silk visible. Spider-to-lens distance was approximately 0.2 m.

Analyses were done in Statistica v9 (StatSoft, Inc.), and data were log (x+1) transformed where necessary to normalize residuals.

Spider observations.—At dusk (19:00–19:30 local time) juveniles emerged from the nest and walked on the web with draglines. Some walked on the thicker frame threads at the edges of the colony web, then stopped and performed tiptoe behavior, releasing very fine silk lines that were visible only in favorable lighting. There appeared to be more than a single line released by an individual. Some juveniles walked with raised abdomen while continuing to release lines. Tiptoe durations were ≤ 30 s and individuals repeatedly alternated between tiptoe behavior, dragline laying and attaching silk to the web. We never observed transitions between spinning cribellate silk and tiptoe behavior. Using strong backlighting, we could see meters-long thin lines streaming upward from the thick frame silk where no spiders were present, suggesting that the spiders had attached the proximal ends of the lines to the web. The movement of these lines indicated that the distal ends remained free.

Wind-tunnel tests.—Juvenile *S. dumicola* released onto the dowel exhibited several behaviors (Table 1): questing; walking; dropping on a dragline, sometimes with release of a thinner, free-ended line; and tiptoeing. The dragline originated from the anterior spinnerets, while the free-ended thin lines, released when tiptoeing (Fig. 1) or when hanging from a dragline, originated from the posterior spinnerets. In some instances, it appeared that more than one line was released from each of the posterior spinnerets.

Overall, 53.2% of juveniles (n = 77) exhibited tiptoe behavior; 14 dropped on a dragline, nine of which also tiptoed. We could not ascertain whether spiders dropping on a dragline always released additional free-ended lines. There was no significant difference in the frequency of tiptoeing in the three nests (χ^2 = 4.435, df = 2, P > 0.1); therefore, in the following analyses data from the three nests were combined.

The first tiptoe behavior in the wind-tunnel tests occurred at 19:50 local time, 20 min after the first individual was tested and during the period when tiptoeing was observed on the webs. The probability of tiptoeing increased significantly with time during the night (logistic regression for binomially distributed data, Wald statistic, W = 10.69, P = 0.001, n = 76). This was also the case when we considered only those spiders engaged in web building when collected (W = 8.397, P = 0.004, n = 28, Fig. 2). Juveniles that were engaging in web repair when they were removed from the web exhibited a somewhat, but not significantly, higher frequency of tiptoe behavior (64.3%) than juveniles collected on prey (39.3%) (Pearson's χ^2 = 3.42, n = 56, df = 1, P = 0.065). Spiders that exhibited tiptoe behavior were significantly smaller in prosoma width than those that did not (Table 2).

Table 1.—Description of behaviors seen in spiders placed on the wooden dowel in the wind tunnel experiments.

Behavior	Description
Quest	Waves legs I in a rotating fashion, either when stationary or while walking with the remaining 6 legs.
Walk	Walks using all eight legs and laying a dragline.
Drop on dragline	Drops on dragline to the substrate, or drops a few cm and hangs on a dragline. When hanging the spider often lets out another line that is caught by the air current.
Tiptoe	Stands on the tips of the tarsi of all 4 pairs of legs, either on the side of the stick or at the top, and releases a silk line that is caught in the air current.

Possible functions of tiptoe behavior in *S. dumicola*.—Social *S. dumicola* disperse to establish new nests by ballooning (long-distance dispersal) and by budding (colony fission) (Schneider et al. 2001; Bilde et al. 2007; Lubin et al. 2009). Adult females engage in aerial dispersal (tiptoeing and releasing silk lines) during the hottest part of the day, under conditions of clear skies and little or no wind (Schneider et al. 2001). The ballooning females mate in their mother colony before dispersal, and after dispersal they establish individual nests that constitute incipient inbred colonies (Henschel 1998; Bilde et al. 2007). We never observed juveniles engaging in aerial dispersal, nor have we ever found new nests established by single juveniles

Figure 1.—Juvenile *S. dumicola* in tiptoe posture while standing on the underside of a wooden dowel mounted in the wind tunnel. Also visible are a) the dragline leading from the spider's anterior spinnerets to the dowel and b) a partially airborne silk line beginning at the posterior spinnerets and following the air flow upward to the right.

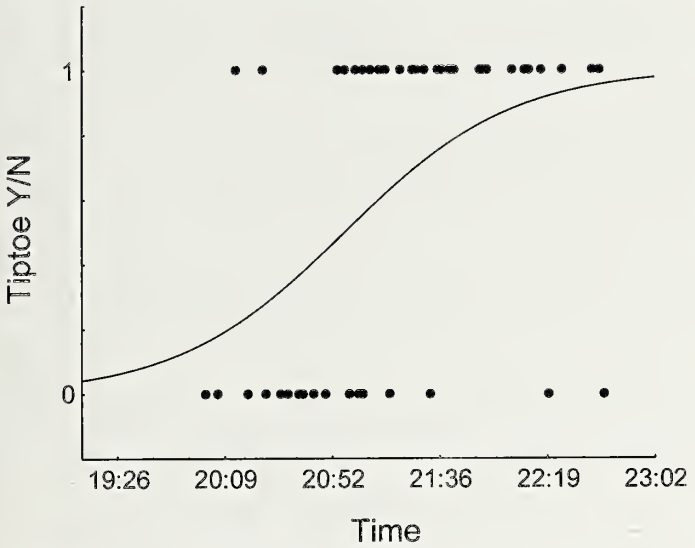

Figure 2.—The probability of tiptoe behavior (yes = 1, no = 0) in juveniles tested at different times of the night. The fitted logistic regression is tiptoe probability = 1/(1+Exp(37.2855-42.6795*x)). Shown here are juveniles collected while engaged in web repair activity (n = 28).

(Schneider et al. 2001; Y. Lubin personal observation). Budding, by contrast, involves movement of a group of large juveniles or subadults a short distance away from the parent nest, and construction of a satellite nest that is initially connected to the parent nest by silk and shares its capture web (Bilde et al. 2007). Budding has not been observed directly, but spiders were seen moving between parent and satellite nests using the connecting web sheets and frame lines (Lubin et al. 2009).

Our observations suggest that tiptoe behavior and release of silk lines by *S. dumicola* juveniles at night are not associated with either ballooning or budding dispersal. First, we observed tiptoeing on the web only at night, and the spiders were all juveniles, thus ruling out ballooning dispersal. Second, spiders that tiptoed on the web released thin lines, but did not become airborne, nor did they turn to grab the lines as in bridging behavior. Third, we found no satellite nests or new groups of juveniles in the vicinity of the study nests during the ten days of observations; thus, budding can be discounted. Finally, in the wind-tunnel experiments, smaller spiders showed a higher frequency of tiptoe behavior; whereas, budding is typically carried out by large juveniles and subadults.

There is some evidence to support the hypothesis that tiptoe behavior in *S. dumicola* is associated with web building. The spiders on the web repeatedly alternated tiptoeing and laying silk on the web; in the wind tunnel, spiders first tiptoed at dusk, coinciding with the start of web building. Finally, there was a non-significant trend of more tiptoeing in spiders collected during web building than during prey capture. We can only speculate about the function of tiptoeing and the release of long, thin, free-ended lines in the context of web building. The upward flowing silk lines might snag on branches above the web and could be used later to construct frame lines for new capture web sheets. However, we did not see spiders testing the released lines or climbing up them, which would argue against such a function. Another possibility is that the lines could intercept small, weakly flying insects that would then drop with the silk into the capture sheets below. Free-ended silk lines will move unpredictably with air currents, and flying insects may be unable to avoid them. Producing cribellate capture-web silk is energetically costly both in material and construction time (Lubin 1986; Opell 1998; Pasquet et al. 1999). By contrast, the thin lines are released rapidly and might increase the probability of intercepting insects early in the evening at minimal cost to the spiders. Arguing against this interpretation is the low probability of insects intercepting such lines and their apparent smoothness. Fine cornstarch and talc did not coat the lines (Y. Lubin & R. Suter personal observation), which suggests also that insects might not adhere to them.

Table 2.—Body size (prosoma width and total body length, in mm) of juveniles that exhibited tiptoe behavior or did not. Standard deviations are in parentheses. Wald statistic and P values of logistic regression for binomially distributed data; variables were log (x+1) transformed.

Variable	Tiptoe behavior		
	Yes	No	Wald W, P
Prosoma width (n=78)	2.6 (0.31)	2.7 (0.30)	W=4.997, P=0.025
Body length (n=79)	7.1 (0.83)	7.5 (0.91)	W=3.409, P=0.064

Wind-tunnel experiments and the function of tiptoeing.—Spiders in the wind tunnel were in an unfamiliar environment and exposed to continuous, directional wind, conditions that differed from those at the web. Tiptoeing and release of silk usually occurred after a spider ran up and down the dowel one or more times, attached dragline silk, and quested intermittently (see Table 1). Spiders that dropped from the dowel on a dragline sometimes continued to the substrate below. These observations suggest that tiptoeing, dropping on a line and the release of aerial lines in the wind tunnel were escape responses. Thus, "pre-dispersal" behaviors are context-dependent and likely have more than one function in *S. dumicola*: adult, mated females adopt them for aerial dispersal, juveniles release silk while web building for reasons yet to be determined, and stressed or disturbed individuals may use them to escape.

Various wind-tunnel experiments have shown consistent differences among individuals in the frequency of tiptoeing and release of lines in relation to developmental, morphological and life-history traits, as well as different environmental conditions (e.g., inbreeding: Bonte 2009; relative leg length: Corcobado et al. 2012; feeding regime: Bonte et al. 2003; microbial endosymbionts: Goodacre et al. 2009). As in our study, these tests show that some individuals are capable of aerial or bridging movements. Furthermore, some are more likely to do so than others, and this correlates with various traits or conditions. However, although a dispersal function is implied, the contexts in which these "pre-dispersal" behaviors occur under natural conditions should be examined rigorously. In our experiments, we found a size-related difference in tiptoe frequency that is at odds with a dispersal function, but consistent with a web-building function.

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SHORT COMMUNICATION

Soil type preference and the coexistence of two species of wandering spiders (*Ctenus amphora* and *C. crulsi*: Ctenidae) in a rainforest in Central Amazonia

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Abstract. The wandering spiders *Ctenus amphora* Mello-Leitão 1930 and *Ctenus crulsi* Mello-Leitão 1930 are sympatric in central Amazonian rainforests; however, the former is more abundant in sandy soils and the latter in clay soils. In previous studies authors suggested that *C. crulsi* is competitively superior on clay soils and avoids sandy soils. Thus, we hypothesized that these species differ in their responses to the soil type. To test this, we placed 37 *C. amphora* and 30 *C. crulsi* in chambers providing two choices (sand or clay) and compared the proportion of observations in each to evaluate both species' preferences. *Ctenus crulsi* significantly preferred clay to sand ($P < 0.01$), while *C. amphora* showed no evidence of preference between two types of soil. We discuss the possible ecological consequences of this difference in behavior. This is the first study that experimentally shows a difference between the responses of spider species to soil type as an explanation of their coexistence.

Keywords: Araneae, behavior, habitat selection, microhabitat

A variety of cues can be used by organisms as proximal stimuli for selecting a habitat (Krebs 1985). Soil type (e.g., sandy, clayey, hydromorphic) may affect many characteristics of the microhabitat, such as litter amount and its associated fauna (De Castillo et al. 2006; Luizão et al. 2007), important resources for ground wandering spiders. However, as far as we know, there are no studies showing that spiders can rely on soil type as a cue to select habitat.

The sympatric spiders *Ctenus amphora* Mello-Leitão 1930 and *C. crulsi* Mello-Leitão 1930 (Araneae: Ctenidae) are among the most abundant medium-sized spiders (mean prosoma length of adults ~7.5–8.9 mm) wandering on the ground in Central Amazonian rainforests (Höfer et al. 1994; Gasnier et al. 2002). However, they differ in patterns of abundance. *Ctenus amphora* is more abundant on relatively dry sandy soils in heath forests or "campinarana", but is also relatively common in clay soil areas with yellow latossol in dense forest vegetation, while *C. crulsi* is predominant in areas of dense forests and is nearly absent in heath forests (Gasnier & Höfer 2001). The diet of both species consists mostly of arthropods, including cockroaches, crickets and other spiders (including other *Ctenus* species: T.R. Gasnier unpublished data) found on the leaf litter. But the diets differ in the high consumption of termites (*Syntermes*: Termitidae) by *C. crulsi* (about 50% of the prey), while termites make up less than 10% of the prey of *C. amphora*. Based on distribution data of *Ctenus* spiders and termites and on the differences in diets, Gasnier et al. (2009) suggested that *C. crulsi*, being more efficient at capturing termites, is competitively superior to *C. amphora* on the clay soil. Furthermore, they suggested that this species avoids sandy soils, where the termites are rare, releasing this habitat to *C. amphora*. Based on the previous studies, we hypothesized that in captivity *C. crulsi* would avoid sandy soil, and *C. amphora* would show no difference in response or would respond positively to sandy soils.

Juvenile *C. amphora* ($n = 37$) and *C. crulsi* ($n = 30$) and samples of sandy and clay soils were collected in August and November 2011 at the "Fazenda Experimental da Universidade Federal do Amazonas" - UFAM (02° 39′41.4″ S, 60° 07′57.5″ W) an area of 3,000 ha of primary rainforest in Central Amazon, Brazil. We used only juvenile *Ctenus* to avoid the influence of difference in activity observed in adult males and females (Salvestrini & Gasnier 2001). We selected spiders with a carapace length greater than 5 mm for inclusion in the experiment, since this is the minimum size sufficient to ensure proper identification to species based on color and design patterns on their bodies (Höfer et al. 1994).

We kept the spiders individually in plastic containers (15 cm diameter × 11 cm height) and subjected them to a constant 12:12 light:dark cycle at ~27°C in the laboratory. We provided water ad libitum and fed the spiders one peanut beetle larva [*Palembus dermestoides* (Fairmaire): Tenebrionidae] once every three days. We deprived the spiders of food for five days prior to the experiment to control their hunger levels. We transported and stored the soil samples for a week after collection in plastic bags until the day of the experiment. We checked the soil samples before the trials to ensure no animals were found within them.

We placed samples of dry clay soil and sandy soil in the plastic containers, 19 cm in diameter × 7 cm height, which had a styrofoam base. The base had a partition 1 cm high and 1 cm wide placed in its center to prevent contact between the two soil types (Fig. 1).

We placed each spider under a plastic vial in the center circle of an arena and left it there to acclimate for 3 min. After this acclimatization period we recorded on which type of soil each spider was found every 10 min between 2010 and 0500 h, making a total of 54 observations for each spider. We used individual samples of soil only once, disposing of them at the end of each set of observations. We tested each spider once. We registered the behavior of 27 spiders during two nights in August 2010 ($n = 17$ *C. amphora*, $n = 10$ *C. crulsi*). Five *C. crulsi* and eight *C. amphora* were observed the first night, and five *C. crulsi* and nine *C. amphora* were observed the second night. We observed 40 other spiders ($n = 20$ *C. amphora*, $n = 20$ *C. crulsi*) during two nights in November 2012, 10 *C. crulsi* and 10 *C. amphora* per night.

For each spider, we recorded the proportion of observations on clay soil (P_{CLAY}) from the total observations on clay and sandy soil. An observation was considered valid when the spider was observed with at least four legs touching one of the soil types and none touching the other soil type.

We used the bootstrap technique to generate confidence intervals of means of P_{CLAY} for each species. Bootstraping allows the calculation of confidence intervals even when the distribution departs from the normal distribution (Efron 1982). In each test, 1000 pseudo-samples

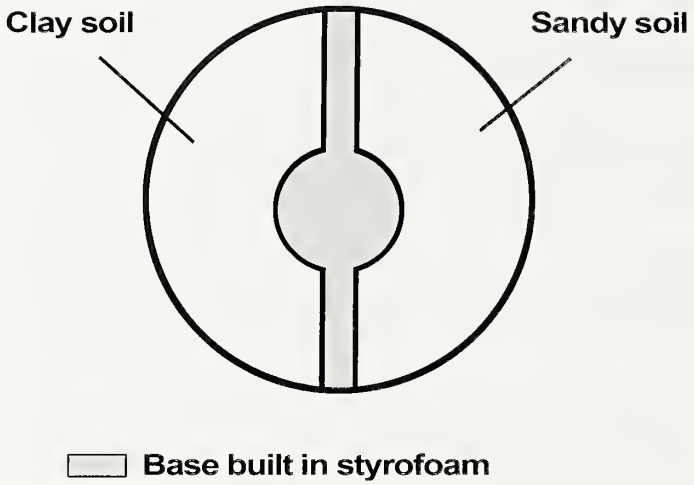

Figure 1.—Testing arena as viewed from above. The bottom was built of styrofoam that had a partition 1 cm high and 1 cm wide (dotted area) to prevent contact between the two soil types. The spider was released in a circular area 5 cm in diameter at the center of the partition.

were generated. The 2.5% inferior and 2.5% superior mean values of the 1000 pseudo-samples were excluded to obtain the 95% confidence interval of mean (CI 95%). The 0.5% inferior and 0.5% superior mean values were excluded to obtain the 99% confidence interval of mean (CI 99%). A confidence interval of P_{CLAY} that does not include 0.5 (the mean expected in the absence of preference) is considered evidence of soil selection. Statistical analyses were done with the software R.12.1 (R Development Core Team 2012).

We deposited voucher specimens in the invertebrate collection of the Instituto Nacional de Pesquisas na Amazônia, Manaus-AM under the numbers INPA-AR 8000–AR-INPA 8005.

The proportion of observations on the clay soil for *C. amphora* (P_{CLAY} = 0.497, CI 95% = 0.435–0.558, CI 99% = 0.420–0.576: Fig. 2) was almost coincident with the expected value in the absence of preference for one of the soil types. The proportion for *C. crulsi* (P_{CLAY} = 0.607, CI 95% = 0.531–0.689, CI 99% = 0.507–0.702) was significantly higher than the expected value in the absence of preference for soil type, indicating a preference for clay by this species.

This result corroborates the hypothesis that *C. crulsi* is able to select areas of clay over sandy soil, which seems to be a proximal cause for their greater abundance on this type of soil. We believe that the choice made by *C. crulsi* is not related to a direct advantage of the soil type, such as a better material for the construction of burrows. A relationship between the distribution of spiders and the soil type has often been found in studies with burrowing spiders (e.g., Hallohan et al. 2000; M'Rabet et al. 2007; Řezáč et al. 2007). However, the two species in this study have never been seen in burrows in the ground in several years of study; apparently, they are using only the leaf litter or tree trunks as shelter (Höfer et al. 1994). Our interpretation is that the type of soil might be a cue for prey occurrence. Whatever the reason, the ability to select the type of soil can be important in this ecosystem where great differences in soil type can be found in nearby places (Chauvel et al. 1987).

The avoidance of sandy soils by *C. crulsi* may also give rise to higher abundance of *C. amphora* in areas with this type of soil. Intraguild predation is probably an important interaction between these species because they prey upon each other, and both species are very abundant on the ground during most months of the year (Gasnier & Höfer 2001). The lack of selection of either of the soils by *C. amphora* observed in this study suggests that its greater abundance in sandy soils is not a result of preference for this soil type as a cue for some important resource used by *C. amphora* in sandy soils. Therefore,

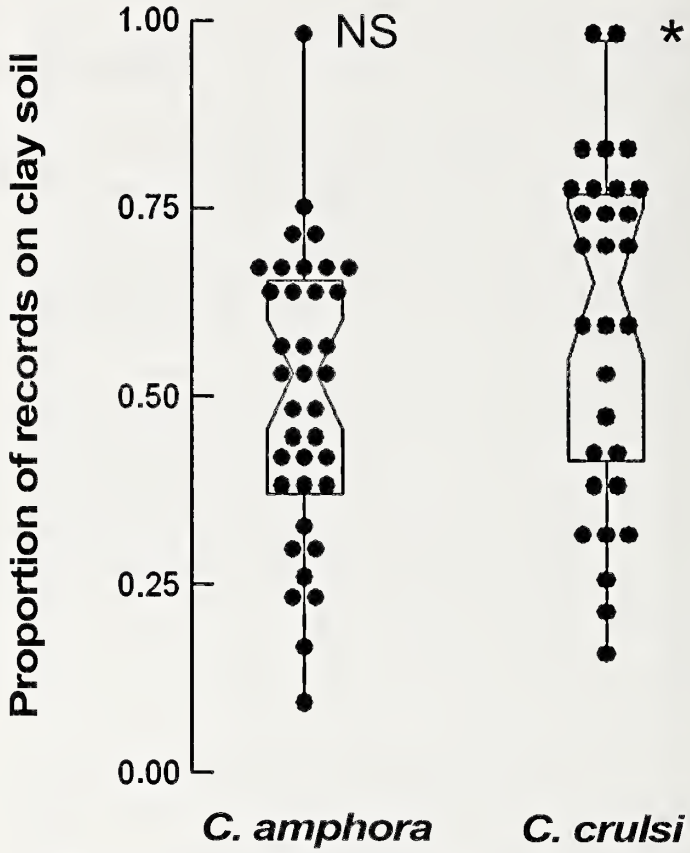

Figure 2.—Proportion of records of the spiders *Ctenus amphora* and *C. crulsi* observed on clay soil among 54 observations at 10-min intervals. The box plot of the data is notched at the median, and widest at the superior and inferior confidence intervals of the median (95%). The overlaying dots represent each spider observed. * indicates $P < 0.01$; NS indicates non-significance.

considering a spatial scale that includes areas of sandy and clay soils, the preference for clay soils by *C. crulsi* may be an important factor contributing to their coexistence. This is the first study to show experimentally a different response to exposure to two soil types by two spider species, which could help to explain their coexistence.

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SHORT COMMUNICATION

Foraging of *Buthus occitanus* (Scorpiones: Buthidae) on shrub branches in an arid area of southeastern Spain

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Abstract. Little is known about the climbing habits of scorpions on plants, despite the interest in this behavior for understanding the connection between above- and below-ground food webs in deserts and to uncover the importance of prey availability and predator avoidance on foraging and habitat selection. Here we report on the foraging of *Buthus* cf. *occitanus* (Amoreux 1789) on shrub branches in an arid area in southeastern Spain. Black-light censuses were carried out within six 50 m × 4 m areas in one full and two new moon nights during September and October 2011. Shrub availability was estimated by counting shrubs in 50 m × 1 m areas within each census area. Results showed that nearly 40% of the scorpions, mostly small, 10–20-mm-long individuals, were found foraging on both inner and outer shrub branches up to 80 cm in height. The probability of finding a scorpion on a shrub was inversely related to scorpion size. Selectivity analysis showed that scorpions selected four shrub species, a result that may be related to prey size and availability. Foraging of *B. occitanus* on shrubs may be favored if this behavior not only allows access to shrub-inhabiting prey, but also reduces predation risk.

Keywords: Above-ground predation, shrub selectivity, size-related behavior

Small species and younger age classes of larger species of scorpions are known to forage both on the ground and on plants, climbing into shrubs and herbs (Polis 1990). Climbing of scorpions on shrubs is interesting for approaching two questions. First, this behavior is useful as a model system to understand how predators link soil and above ground food webs (Wardle et al. 2004; González-Megías et al. 2011), especially in arid ecosystems where scorpions are usually abundant. Second, despite the interest in this behavior for understanding the relative importance of resource availability and predation risk on foraging and habitat selection (Brown & O'Connell 2000), use of shrubs by scorpions has received little attention. Only a few papers have addressed this question studying a buthid species, *Centruroides vitattus* (Say 1863) (Brown & O'Connell 2000, McReynolds 2008). Here, we report the foraging of *Buthus* cf. *occitanus* (Amoreux 1789) on shrub branches in arid areas of the Guadix-Baza Basin (Granada province, southeastern Spain). The *B. occitanus* species complex (see Fet 2010; Rossi 2012) is comprised of large species showing flexibility in foraging behavior (Skutelsky 1995). The foraging of *Buthus occitanus* on branches of shrubs has been reported in the Negev Desert (Skutelsky 1996) and on small Mediterranean islands (Castilla & Pons 2007). In the Negev, Skutelsky (1996) found that 3% of the adult and 40% of the juvenile scorpions were observed on the outer branches of bushes. However, no other papers have described the climbing behavior of *Buthus occitanus* on shrubs, and no preeise information has been provided on 1) whether there are differences among size classes in climbing on shrubs, 2) selection for certain shrub species and whether it may be related to prey availability and 3) where the scorpions are located (height, inner/outer branches) on the shrubs.

Observations were made at Barranco del Espartal, a location in the arid Guadix-Baza Basin (Granada province, southeastern Spain). The site is an occasional watercourse with a gypsum loam substrate. The vegetation is an open shrubsteppe (58% bare soil, 41% shrub cover) dominated by *Artemisia* and *Salsola* shrubs, *Retama* bushes and *Stipa* tussock grasses (see Doblas et al. 2009 for a more detailed description of the study site). Sampling was carried out by exhaustive search using a black light in a total of six randomly selected 50 m × 4 m straight-line transects in plain sites around the dry riverbed. We surveyed scorpions on two new moon nights in September and one full moon night in October 2011 from 21:00 to 01:00. Each night we surveyed two different 50 m × 4 m transects, for a total of four transects in the two new moon nights and two transects in the full moon night. We measured the length (chelicerae to telson) of each scorpion (both on the ground and on shrubs) directly in the field. Scorpions were restrained in a thick, transparent plastic bag and their tails were straightened using a pair of forceps, measuring the total length of each individual with a small metal ruler. In addition, we recorded the type of shrub, the height of the scorpion and the scorpions position relative to the shrubs outside envelope (10 cm outer or > 10 cm inner branches). After measurement, each individual was released where it was found.

We used a Chi-square test to compare 1) the frequency distribution of scorpion size categories (>10–20 mm, >20–30 mm, >30–40 mm, >40–50 mm, > 50–60 mm, > 60 mm), and 2) the difference in frequency of scorpions on shrubs and on the ground between the two new moon nights and the full moon night. To test whether the presence of scorpions on shrubs or on the ground (binary categorical dependent variable) was correlated with scorpion size (continuous independent variable), we carried out a logistic regression (Quinn & Keough 2002) using the Statistica 7.1 package (StatSoft 2005). To analyze whether scorpions selected different types of shrubs, we counted the number of shrubs of each type in a 50 m × 1 m area in the center of each transect surveyed. Selectivity was quantified using the *Wi* Savage's index. This index is the ratio between the proportion of resources used and the proportion of available resources in the environment, with index values > 1 indicating preference and index values < 1 avoidance (Krebs 1999). Significance of selection was evaluated by the χ^2 test (Krebs 1999), with a posteriori correction of significance by the sequential Bonferroni procedure (Holm 1979).

In all, 148 scorpions ranging from 13 to 65 mm length were recorded during the surveys. Scorpion density was similar on the two new moon nights (23.0 ± 4.6 individuals/200 m^2, range 11–33 individuals/200 m^2) and the full moon night (22 and 35 individuals/200 m^2). Neither size frequency distribution ($\chi^2 = 5.44$, $P = 0.25$, $n = 148$) nor frequency of scorpions on shrubs and on the ground ($\chi^2 = 3.33$, $P = 0.07$, $n = 148$) showed differences between the two new

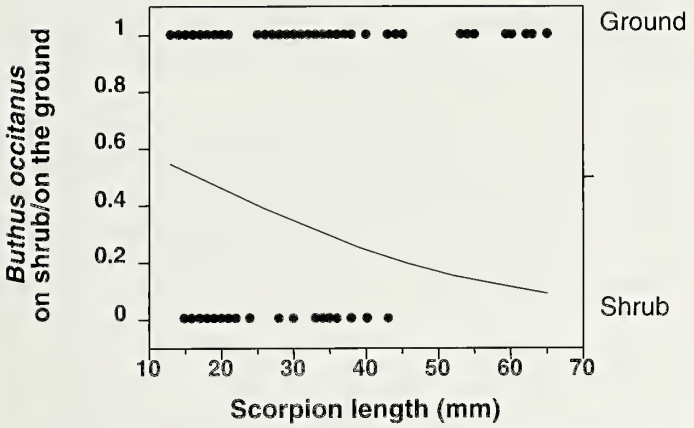

Figure 1.—Scatterplot of *Buthus* cf. *occitanus* observations on shrubs and on the ground in relation to body length, and predicted probability curve from the logistic regression model.

moon nights and the full moon night. Because data from the two new moon nights and the full moon night did not differ significantly, subsequent analyses were carried out pooling all surveys.

The population structure of *B.* cf. *occitanus* in the study site was dominated by 10–20 mm individuals, which comprised 54.7% of the total scorpions observed. In total, 39.9% of all individuals were found on shrub branches, although the proportion of individuals foraging on shrubs and on the ground varied with size: 46% of the smallest scorpions (10–20 mm length) occurred on shrubs, while 34% of the individuals measuring > 20 mm to 50 mm length and none of the individuals > 50 mm length were observed on shrubs. A logistic regression showed that the probability of finding a scorpion on a shrub significantly decreases as size increases, while the probability of finding a scorpion on the ground increases with body length (intercept: estimate = 0.615 ± 0.445, Wald χ^2 = 1.909, P = 0.167, df = 1; scorpion size: estimate = −0.043 ± 0.017, Wald χ^2 = 6.448, P = 0.011, df = 1) (Fig. 1).

Scorpions did not use shrubs randomly, but showed a significant selectivity for some shrub species (*Retama sphaerocarpa*, *Gypsophila struthium*, *Ononis tridentata* and *Lepidium subulatum*: Table 1). Scorpions occupied shrub branches 19.2 ± 4.5 cm high on average, most individuals (45%) occurring 10–20 cm high in the plants, but several individuals were above 50 cm and up to 80 cm. Individuals on shrubs were on both outer (53%) and inner (47%) branches of the plants. Scorpions remained still on the branches, usually facing down with pedipalps open (Fig. 2), attacking arthropods when they were close by (sit-and-wait foraging). During the surveys, we observed three scorpions successfully capturing prey (2 moths, 1 Cicadellidae) and four other scorpions with captured prey (2 moths, 1 *Clubiona* sp.

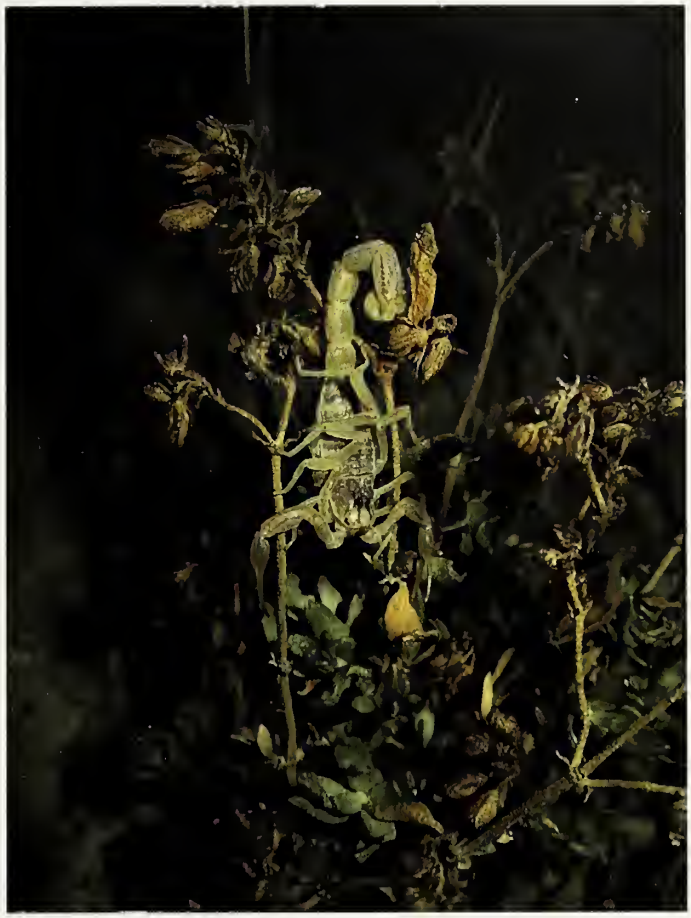

Figure 2.—"Sit and wait" foraging of *Buthus* cf. *occitanus* on a shrub, *Helianthemum squamatum*.

epiphytic spider, 1 *B.* cf. *occitanus* scorpion) on shrub branches, while we only observed one scorpion unsuccessfully attacking a moth and one scorpion with a captured *Dicranocephalus* sp. (Heteroptera: Stenocephalidae) on the ground.

Our data showed that climbing behavior of *B.* cf. *occitanus* was related to scorpion size, small individuals having a higher probability of climbing on shrubs than larger scorpions. The higher proportion of juveniles than adults on vegetation has been previously indicated for both *B. occitanus* (Skutelsky 1996) and other scorpion species (Polis 1990; Brown & O'Connell 2000). Because the climbing behavior of *B. occitanus* is related to scorpion size, the proportion of individuals foraging on shrubs may vary seasonally due to phenological changes

Table 1.—Shrub selectivity (Savage's index, W_i) by *Buthus* cf. *occitanus*. * Significant positive selection ($P < 0.05$, χ^2 test after sequential Bonferroni correction).

Shrub	% used	% available	W_i	S.E. (W_i)	χ^2
Artemisia spp.	19.51	29.59	0.66	0.32	1.11
Gypsophila struthium	2.44	0.46	**5.36***	1.14	14.54
Helianthemum violaceum	2.44	1.21	2.01	1.05	0.93
Helianthemum squamatum	17.07	21.24	0.80	0.35	0.31
Lepidium subulatum	14.63	7.13	**2.05***	0.40	6.82
Ligeum spartum	7.32	8.65	0.85	0.57	0.07
Ononis tridentata	2.44	0.15	**16.08***	1.40	115.21
Retama sphaerocarpa	12.20	0.30	**40.20***	0.82	2279.72
Salsola vermiculata	4.88	7.13	0.68	0.70	0.20
Stipa spp.	4.88	4.40	1.11	0.71	0.02
Thymus zygis	12.20	19.73	0.62	0.43	0.80

in the age (and size) structure of scorpion populations (e.g., Warburg & Polis 1991; Brown & O'Connell 2000).

Foraging by scorpions on shrubs has been explained as a behavior to avoid predation, especially by other scorpions (Polis 1980; Polis & McCormick 1987), and also to exploit higher prey availability on plants than on the ground (Polis 1990; Skutelsky 1996; Brown & O'Connell 2000). On the one hand, our observations suggest that foraging on shrubs could be more profitable than on the ground. The fact that in the study site most arthropods on shrubs are much smaller (1.3 mg average dry weight) than on the ground (16.2 mg average dry weight: Sánchez-Piñero 1994) may be an important factor related to the higher frequency of small scorpions on shrubs. Also, previous studies of shrub canopy arthropods carried out in the study area by means of beating (Sánchez Piñero 1994; Sánchez-Piñero et al. unpublished data) allow us to hypothesize that selection of shrubs by scorpions is related to prey availability. Thus, available information showed that *Gypsophila struthium* and *Retama sphaerocarpa* had 4 and 2.5 times higher abundances of canopy arthropods, respectively, than most other shrubs in the study area. The fact that *R. sphaerocarpa* also exhibited very high densities of night-active insects during our *B.* cf. *occitanus* surveys also supports the idea that prey availability is related to shrub selection by the scorpions. *Lepidium subulatum* and *Ononis tridentata* were the only two flowering shrub species during our scorpion surveys, and we observed higher numbers of night-active insects (especially moths and homopterans flying near the flowers) in these plants than about other shrubs. The lack of significant selection for other shrubs may be related to their low canopy arthropod abundance in the study area (Sánchez-Piñero 1994).

Although we have observed three instances of *B. occitanus* capturing prey on shrubs, we do not know whether scorpions also climb onto shrubs to eat prey captured on the ground, as suggested for *Centruroides vittatus* (McReynolds 2008). Thus, foraging of *B. occitanus* on shrubs may be favored if this behavior not only allows access to shrub-inhabiting prey, but also reduces both inter- and intraspecific predation risks (Skutelsky 1996; Brown & O'Connell 2000; McReynolds 2008). More research will be necessary to evaluate whether prey availability is higher and risks of predation and cannibalism lower on shrubs than on the ground and to uncover the factors related to scorpion foraging on shrubs.

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INSTRUCTIONS TO AUTHORS
(revised February 2013)

General: The *Journal of Arachnology* publishes scientific articles reporting novel and significant observations and data regarding any aspect of the biology of arachnid groups. Feature articles and short communications must be scientifically rigorous and report substantially new information. Submissions that are overly narrow in focus (e.g., local faunal lists, descriptions of a second sex or of a single species without additional discussion of the signifieance of this information), have poorly substantiated observational data, or that present no new information will not be considered. Book reviews will not be published.

Manuscripts must be in English and should be prepared in general accordance with the current edition of the *Council of Biological Editors Style Manual* unless instructed otherwise below. Use the active voice throughout. Authors should consult a recent issue of the *Journal of Arachnology* for additional points of style. Manuscripts longer than three printed journal pages (12 or more double-spaced manuscript pages) should be prepared as Feature Articles, shorter papers as Short Communications. Review Articles will be published from time to time. Suggestions for review articles may be sent to the Managing Editor. Unsolicited review articles are also welcomed. All review articles will be subject to the same review process as other submissions.

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The Managing Editor will acknowledge receipt of the manuscript, assign it a manuscript number and forward it to an Associate Editor for the review process. Correspondence relating to manuscripts should be directed to the Associate Editor and should include the manuscript number. If the manuscript is accepted, the author will be asked to submit the final copy electronically to the Associate Editor. Submission of final illustrations is detailed below. Authors are expected to return revisions promptly. Revised manuscripts that are not returned in a reasonable time period (no longer than six months for minor revisions and one year for major revisions) will be considered new submissions.

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FEATURE ARTICLES

Title page.—The title page includes the complete name, address, and telephone number of the corresponding author; a FAX number and electronic mail address if available; the title in sentence case, with no more than 65 characters and spaces per line in the title; each author's name and address; and the running head.

Running head.—The author's surname(s) and an abbreviated title should be typed in all capital letters and must not exceed 60 characters and spaces. The running head should be placed near the top of the title page.

Abstract.—Length: ≤ 250 words for Feature Articles; ≤ 150 words for Short Communications.

Keywords.—Give 3–5 appropriate keywords or phrases following the abstract. Keywords should not duplicate words in the title.

Text.—Double-space text, tables, legends, etc. throughout. Three levels of heads are used.

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Citation of taxa in the text: Include the complete taxonomic citation (author & year) for each arachnid taxon when it first appears in the abstract and text proper. For Araneae, this information can be found online at http://research.amnh.org/iz/spiders/catalog/. For example, *Araneus diadematus* Clerck 1757. Citations for seorpions can be found in the *Catalog of the Scorpions of the World (1758–1998)* by V. Fet, W.D. Sissom, G. Lowe & M.E. Braunwalder. Citations for pseudoscorpions can be found at http://www.museum.wa.gov.au/catalogues/pseudoscorpions/. Citations for some species of Opiliones can be found in the *Annotated Catalogue of the Laniatores of the New World (Arachnida, Opiliones)* by A.B. Kury. Citations for other arachnid orders can be found in *Catalogue of the Smaller Arachnid Orders of the World* by M.S. Harvey.

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Huber, B.A. & W.G. Eberhard. 1997. Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). Canadian Journal of Zoology 74:905–918.

Krafft, B. 1982. The significance and complexity of communication in spiders. Pp. 15–66. *In* Spider Communication: Mechanisms and Ecological Significance. (P.N. Witt & J.S. Rovner, eds.). Princeton University Press, Princeton, New Jersey.

Platnick, N.I. 2011. The World Spider Catalog, Version 12.0. American Museum of Natural History, New York. Online at http://research.amnh.org/iz/spiders/catalog/

Roewer, C.F. 1954. Katalog der Araneae, Volume 2a. Institut Royal des Sciences Naturelles de Belgique, Bruxelles.

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Short Communications are usually limited to three journal pages, including tables and figures (11 or fewer double-spaced manuscript pages including Literature Cited; no more than 2 small figures or tables). Internal headings (METHODS, RESULTS, etc.) are omitted. Short communications must include an abstract and keywords.

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